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Kubota et al.

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[45] **Date of Patent: *Nov. 3, 1998**

[54] **DNA ENCODING ENZYME, RECOMBINANT DNA AND ENZYME, TRANSFORMANT, AND THEIR PREPARATIONS AND USES**

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[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,716,813.

[21] Appl. No.: **961,240**

[22] Filed: **Oct. 30, 1997**

Related U.S. Application Data

[60] Continuation of Ser. No. 607,321, Feb. 26, 1996, Pat. No. 5,716,813, which is a division of Ser. No. 399,646, Mar. 7, 1995, Pat. No. 5,556,781.

[30] **Foreign Application Priority Data**

Mar. 7, 1994 [JP] Japan 6-59834
Mar. 7, 1994 [JP] Japan 6-59840

[51] **Int. Cl.**[6] **C12N 9/24**; C12N 9/26; C12N 1/02; C13J 1/00

[52] **U.S. Cl.** **435/96**; 435/200; 435/201; 435/276

[58] **Field of Search** 435/96, 200, 201, 435/276

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,521,252	6/1985	Miyake et al.	127/46.3
5,455,168	10/1995	Maruta et al.	435/201
5,472,863	12/1995	Maruta et al.	435/200

FOREIGN PATENT DOCUMENTS

0555540	12/1992	European Pat. Off. .
0628630	6/1994	European Pat. Off. .
0154485	12/1975	Japan .
0023799	2/1983	Japan .
0072598	4/1983	Japan .
0216695	12/1983	Japan .
2106912	4/1983	United Kingdom .

OTHER PUBLICATIONS

E.M. Southern *Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis,* J. Mol. Biol., vol. 98, pp. 503–517, 1975.

Enzyme Kinetics of Amylases and Related Enzymes, Handbook of Amylases and Related Enzymens, pp. 1–9, 1988.

Maruta et al., Biochimica et Biophysica Acta, "Cloning and Sequencing of Trehalose Biosynthesis Genes from Arthrobacter sp. Q36", No. 1289, 1996, pp. 10–13.

U.K. Laemmli, *Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4,* Nature, vol. 227, pp. 680–685, 15 Aug. 1970.

J. Sambrook et al., *Molecular Cloning a Laboratory Manual,* Second Edition, published by Cold Spring Harbor Laboratory Press, 1989, pp. 9.47–9.58 and 11.45–11.54.

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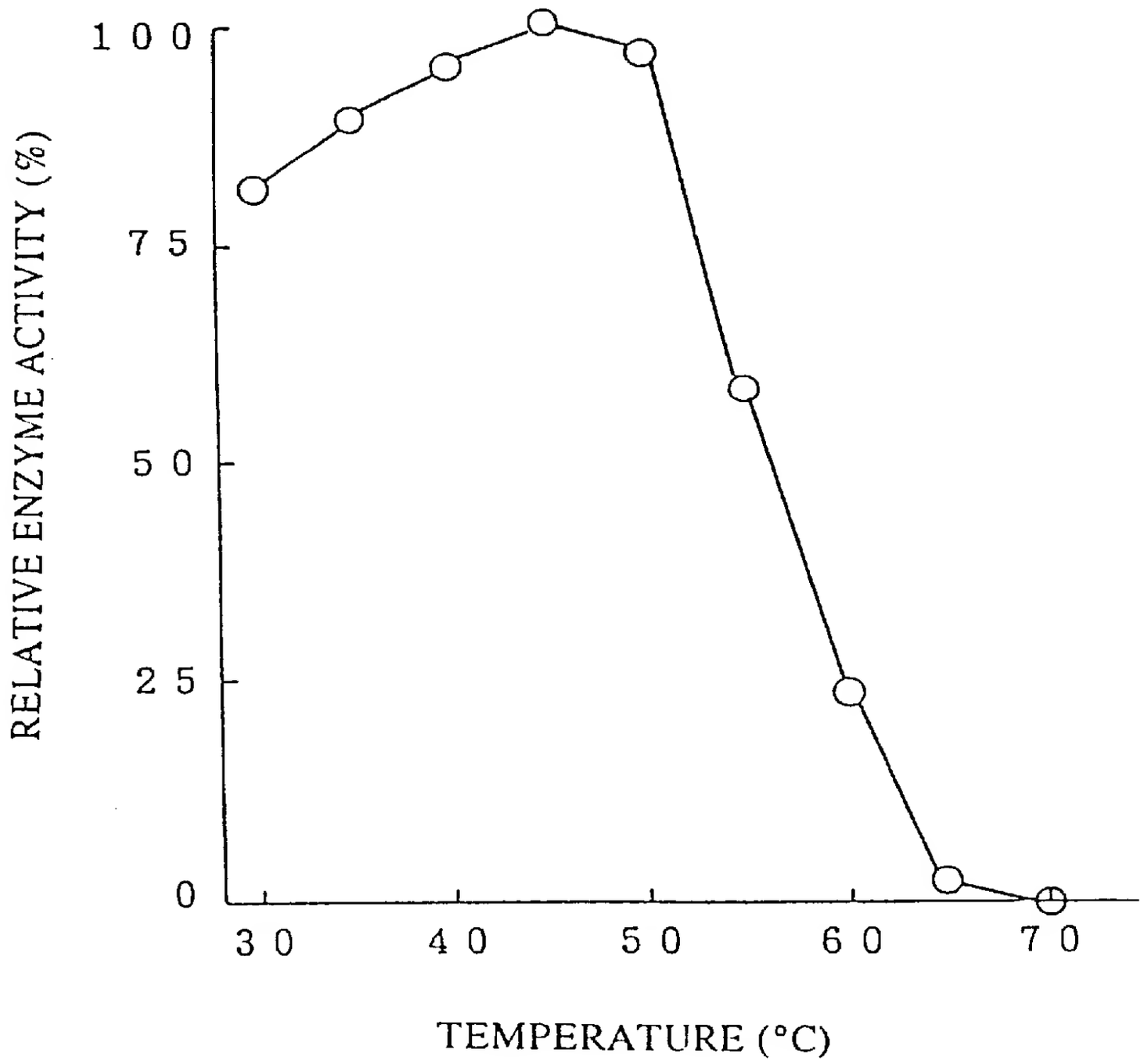
Assistant Examiner—Lisa J. Hobbs
Attorney, Agent, or Firm—Browdy and Neimark

[57] **ABSTRACT**

Disclosed are a DNA encoding an enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, recombinant DNA and enzyme, transformant, and their preparations and uses. These facilitate the industrial-scale production of trehalose with a relative easiness and low cost, and trehalose thus obtained can be satisfactorily used in a variety of food products, cosmetics and pharmaceuticals.

7 Claims, 9 Drawing Sheets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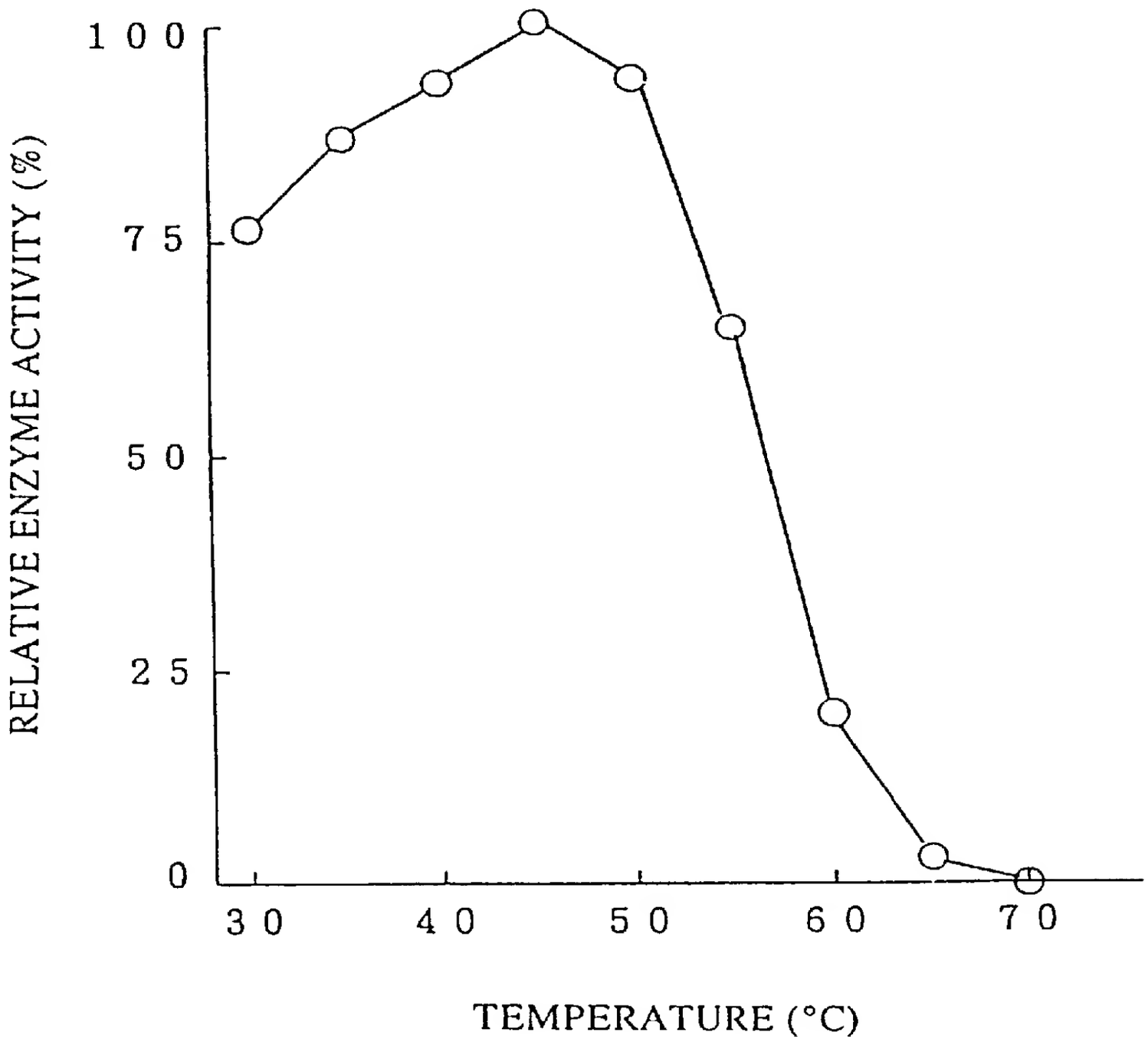
TEMPERATURE (°C)

FIG. 1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RELATIVE ENZYME ACTIVITY (%)
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FIG. 2

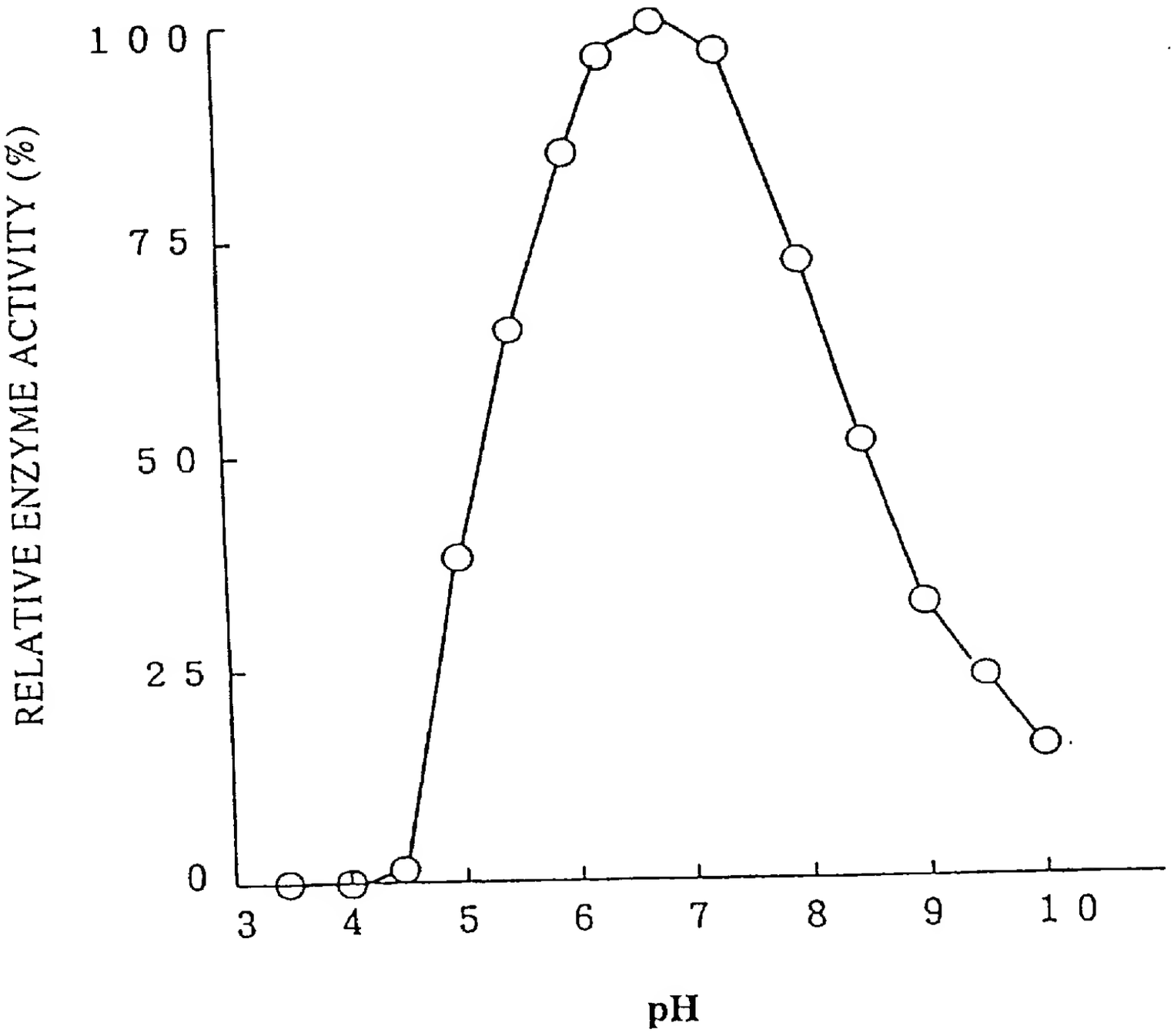


FIG. 3

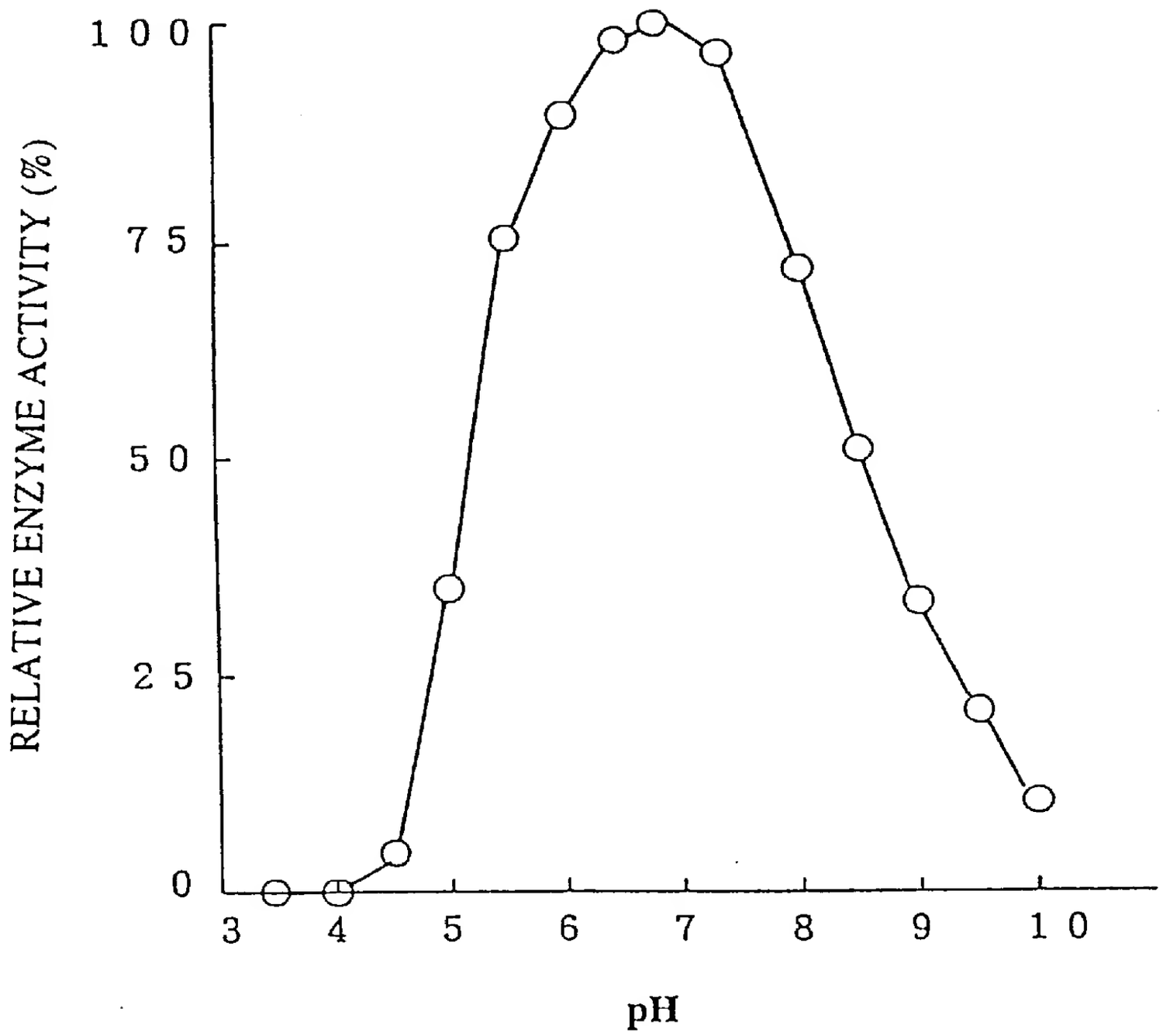
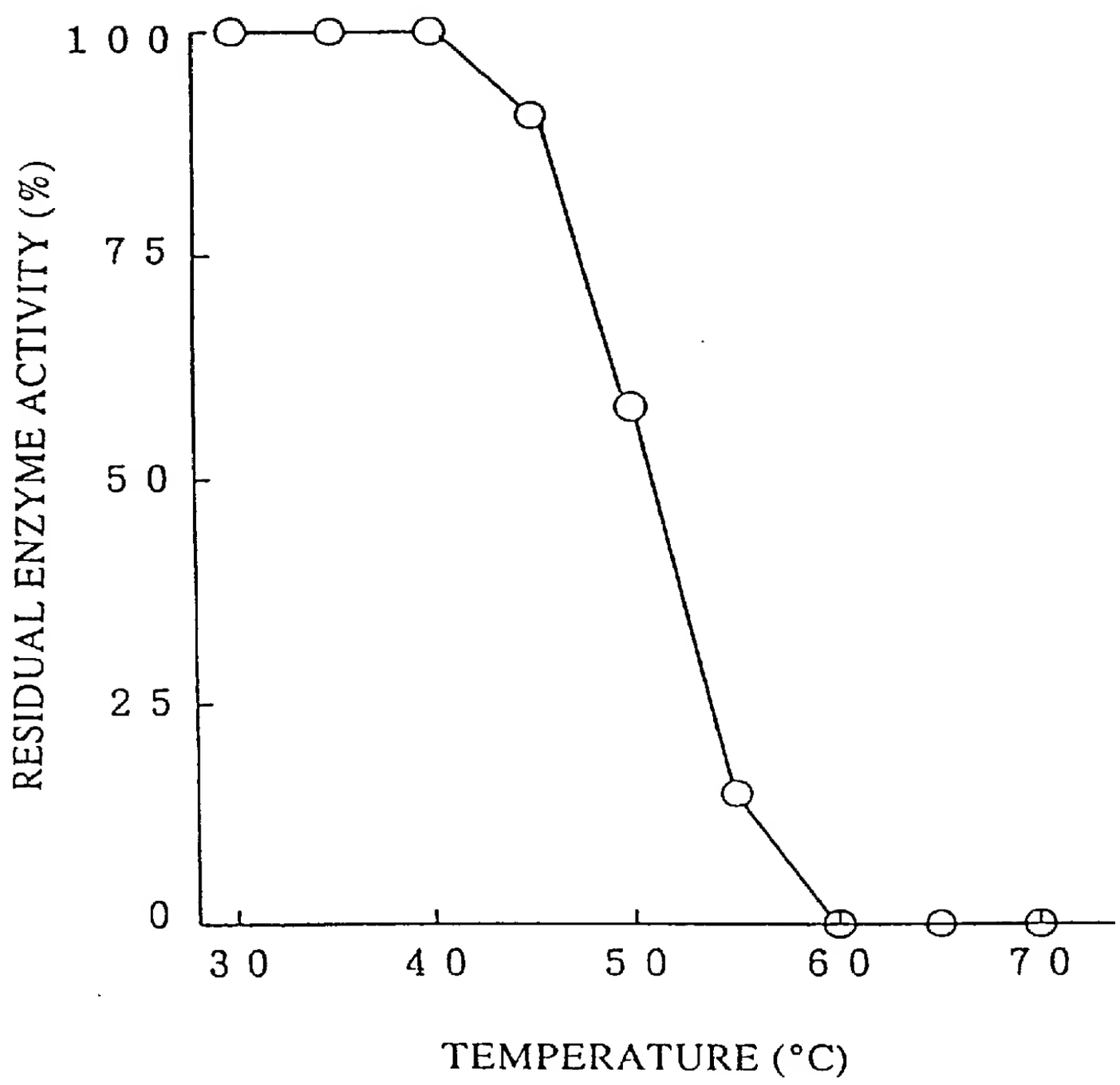


FIG. 4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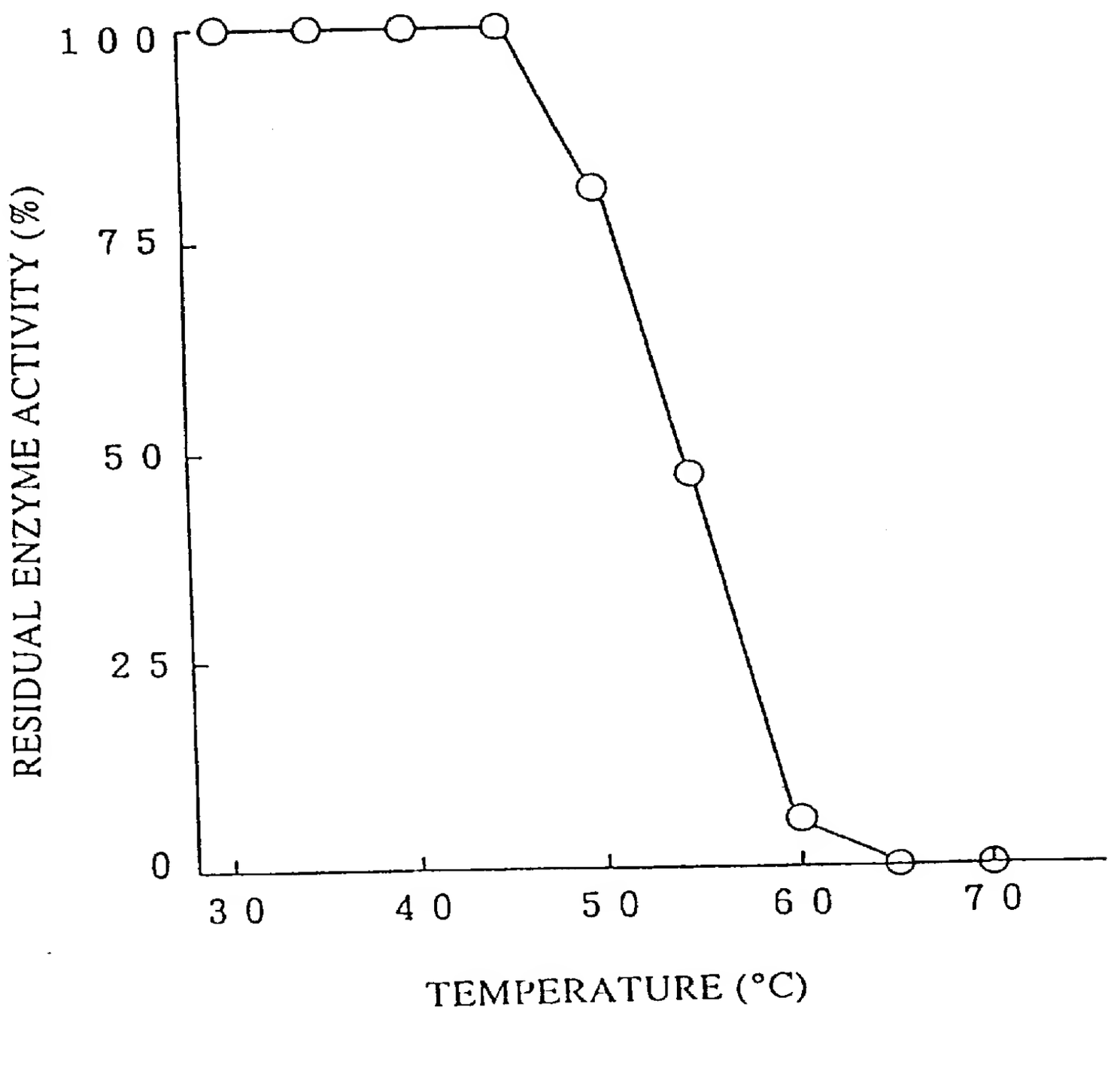
TEMPERATURE (°C)

FIG. 5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RESIDUAL ENZYME ACTIVITY (%)
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40
50
60
70
TEMPERATURE (°C)

FIG. 6

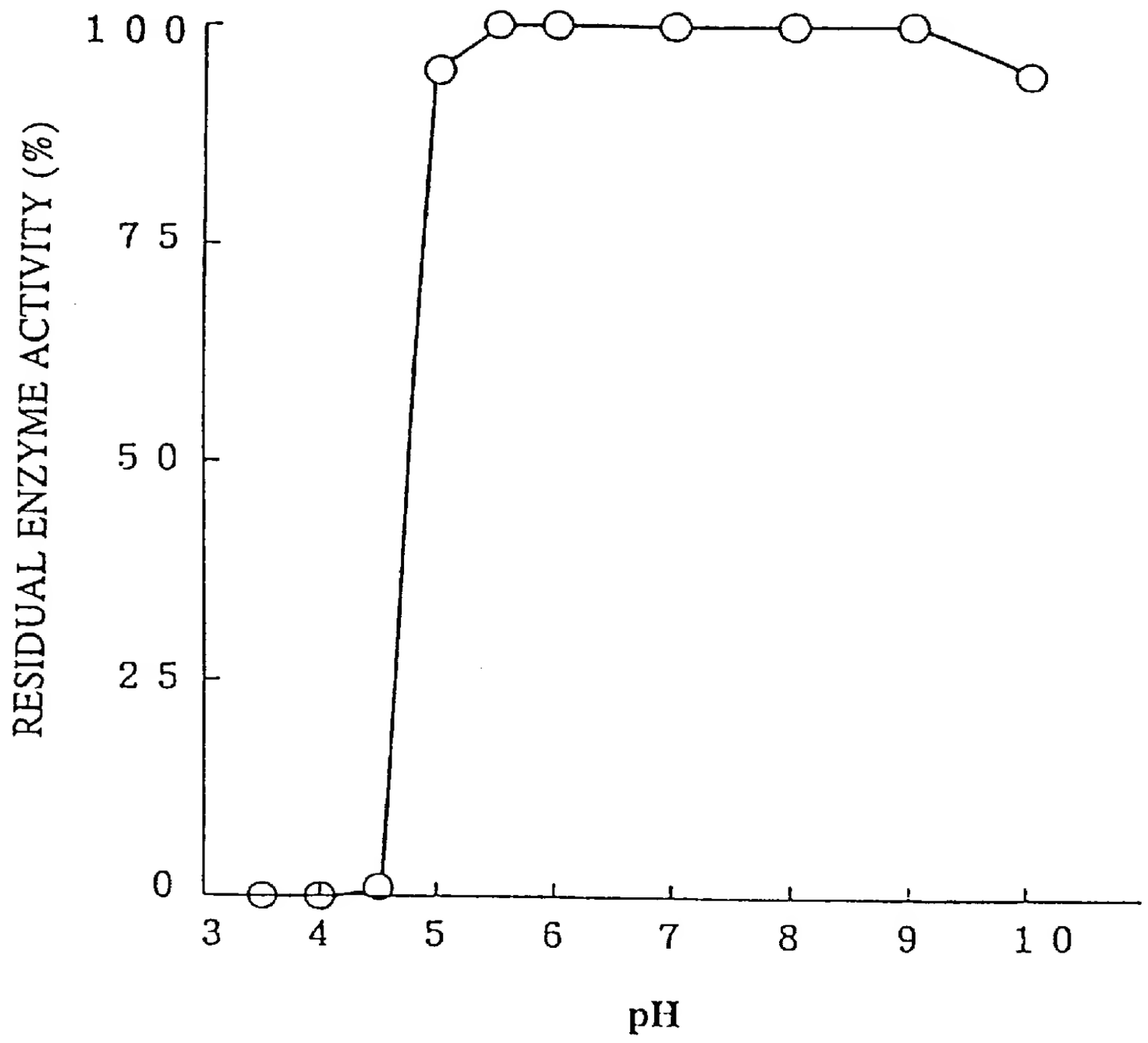


FIG. 7

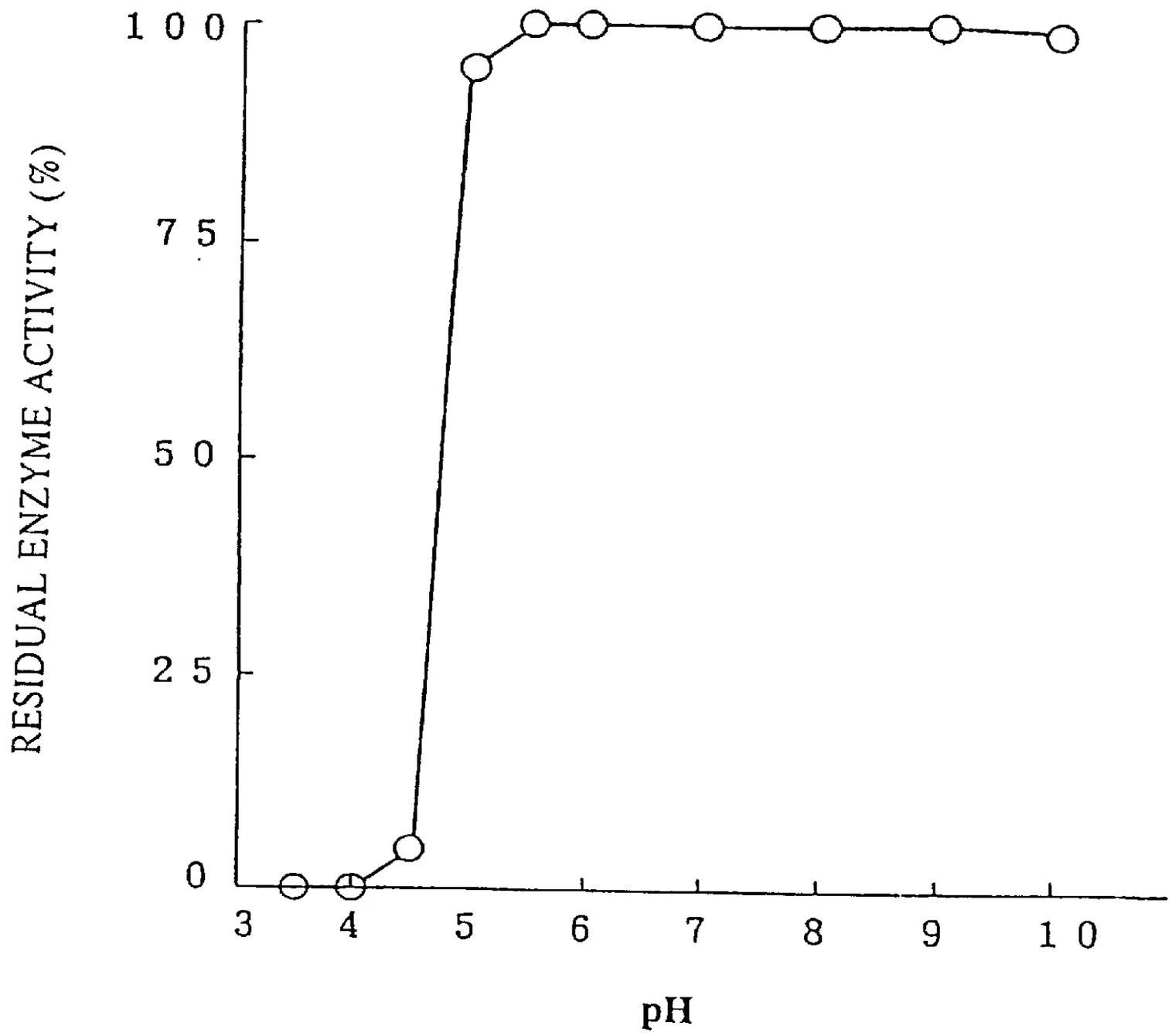
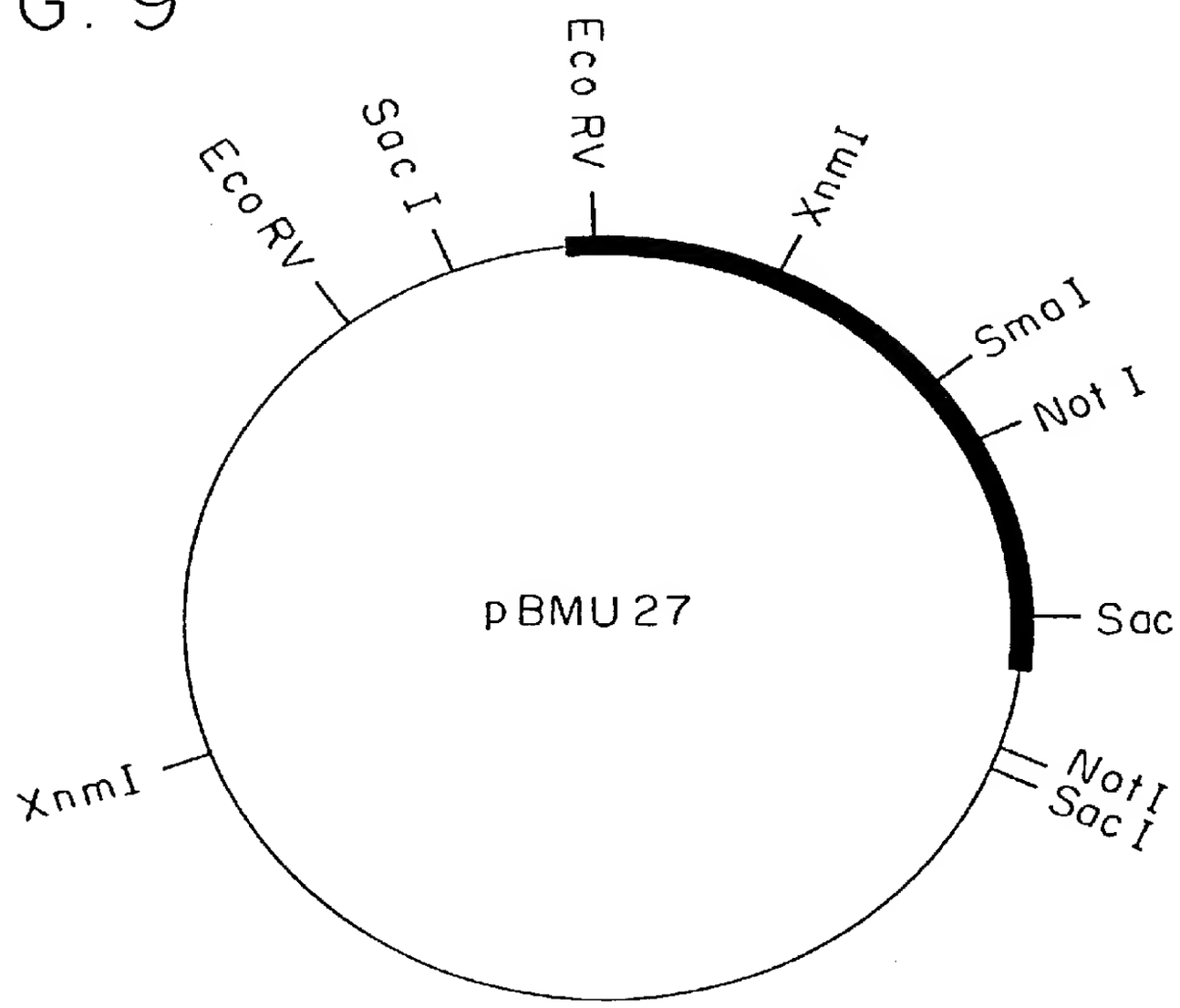


FIG. 8

FIG. 9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Xnm I
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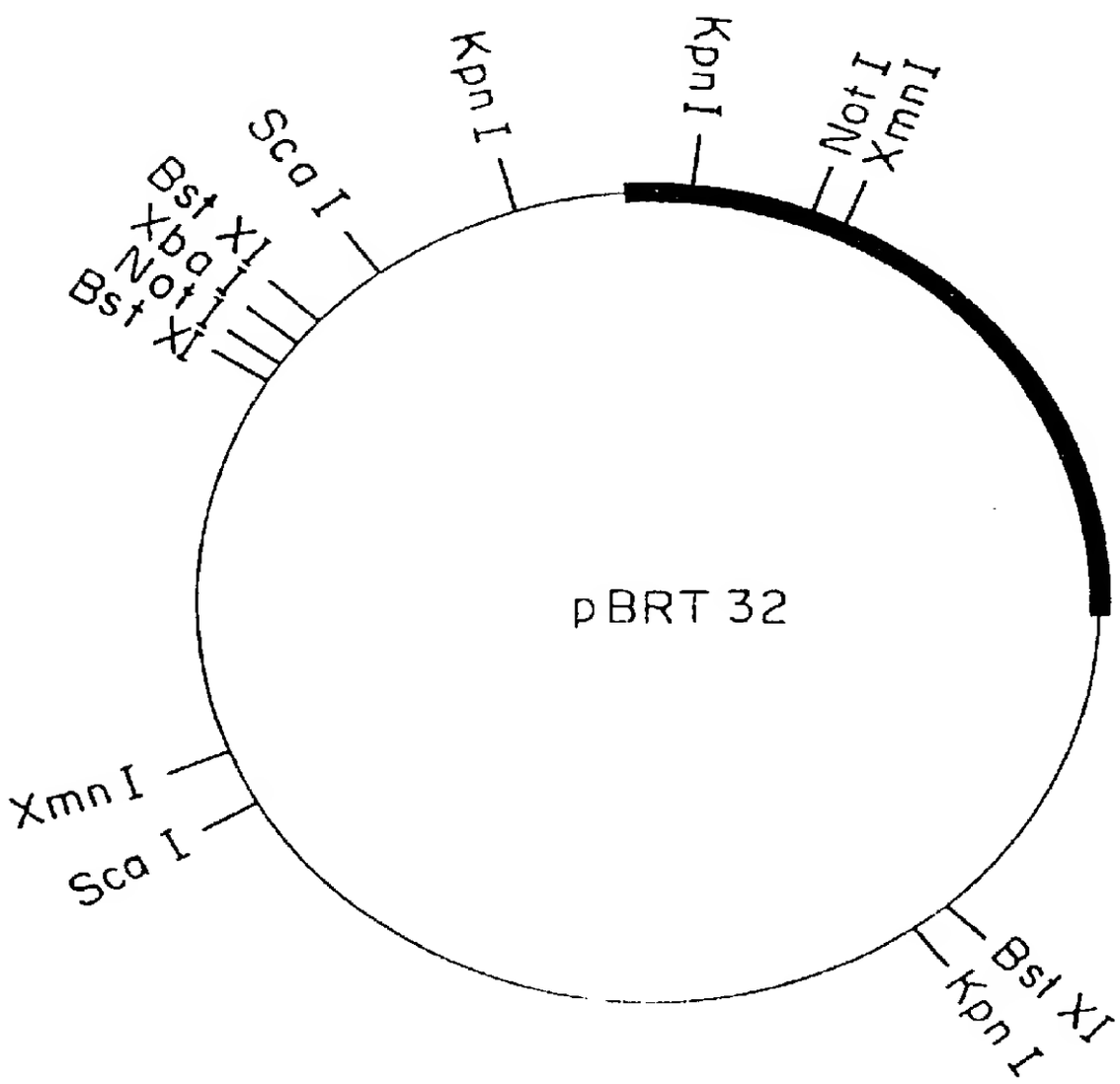
FIG. 10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pBRT 32

DNA ENCODING ENZYME, RECOMBINANT DNA AND ENZYME, TRANSFORMANT, AND THEIR PREPARATIONS AND USES

This is a continuation of parent application Ser. No. 08/607,321 filed Feb. 26, 1996, now issued as U.S. Pat. No. 5,716,813, which is a divisional of application Ser. No. 08/399,646, filed Mar. 7, 1995, now issued as U.S. Pat. No. 5,556,781.

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The present invention relates to a novel DNA encoding an enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, recombinant DNA containing the same, and a transformant, and further relates to a recombinant enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, as well as to preparations and uses thereof.

2. Description Of The Prior Art

Trehalose is a disaccharide which consists of 2 glucose molecules which are linked together with their reducing groups, and, naturally, it is present in bacteria, fungi, algae, insects, etc., in an extremely small quantity. Having no reducing residue within the molecule, trehalose does not cause an unsatisfactory browning reaction even when heated in the presence of amino acids or the like, and because of this it can sweeten food products without fear of causing unsatisfactory coloration and deterioration. Trehalose, however, is far from being readily prepared in a desired amount by conventional methods, and, actually, it has not scarcely been used for sweetening food products.

Conventional methods are roughly classified into 2 groups, i.e. the one using cells of microorganisms and the other employing a multi-enzymatic system wherein enzymes are allowed to act on saccharides. The former, as disclosed in Japanese Patent Laid-Open No.154,485/75, is a method which comprises allowing to grow microorganisms such as bacteria and yeasts in a nutrient culture medium, and collecting trehalose from the proliferated cells in the resultant culture. The latter, as disclosed in Japanese Patent Laid-Open No.216,695/83, is a method which comprises providing maltose as a substrate, allowing a multi-enzymatic system using maltose- and trehalose-phosphorylases to act on maltose, and isolating the formed trehalose from the reaction system. Although the former facilitates the growth of microorganisms with a relative easiness, it requires a sequentially-complicated step for collecting trehalose from the microorganisms which contain at most 15 w/w % trehalose, on a dry solid basis (d.s.b.). While the latter enables the separation of trehalose itself with a relative easiness, but it is theoretically difficult to increase the trehalose yield by allowing enzymes to act on substrates at a considerably-high concentration because the enzymatic reaction per se is an equilibrium reaction of 2 different types of enzymes and the equilibrium point constantly inclines to the side of forming glucose phosphate.

In view of the foregoing, the present inventors energetically screened enzymes which form saccharides having a trehalose structure from amylaceous saccharides, and found that microorganisms such as those of the spices *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36 produce an absolutely novel enzyme which forms non-reducing saccharides having

a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of 3 or higher. Before or after this finding, it was revealed that such non-reducing saccharides are almost quantitatively hydrolyzed into trehalose and glucose and/or maltooligosaccharides by other enzymes produced from the same microorganisms of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36. Since the combination use of such enzymes enables to form a desired amount of trehalose with a relative easiness, the aforementioned objects relating to trehalose would be completely overcome. Insufficient producibility of such enzymes by the microorganisms results in a drawback that a relatively-large scale culture of the microorganisms is inevitable to industrially produce trehalose and/or non-reducing saccharides having a trehalose structure as an end unit.

Recombinant DNA technology has made a remarkable progress in recent years. At present, even an enzyme, whose total amino acid sequence has not yet been revealed, can be readily prepared in a desired amount, if a gene encoding the enzyme was once isolated and the base sequence was decoded, by preparing a recombinant DNA containing a DNA which encodes the enzyme, introducing the recombinant DNA into microorganisms or cells of plants or animals, and culturing the resultant transformants. Under these circumstances, urgently required are the finding of genes which encode these enzymes and the elucidation of their base sequences.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a DNA which encodes an enzyme that releases trehalose from non-reducing saccharides having a trehalose structure as an end unit.

It is a further object of the present invention to provide a replicable recombinant DNA containing the aforesaid DNA.

It is yet another object of the present invention to provide a transformant which is prepared by introducing the recombinant DNA into an appropriate host.

It is a further object of the present invention to prepare the aforesaid enzyme by the application of the recombinant DNA technology.

It is a further object of the present invention to provide a preparation of the enzyme.

It is a further object of the present invention to provide a method for converting non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The first object of the present invention is attained by a DNA which encodes an enzyme that releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The second object of the present invention is attained by a replicable recombinant DNA which contains the aforesaid DNA and a self-replicable vector.

The third object of the present invention is attained by a transformant prepared by introducing the aforesaid self-replicable vector into an appropriate host.

The fourth object of the present invention is attained by a recombinant enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The fifth object of the present invention is attained by a process to produce the recombinant enzyme comprising

culturing a transformant capable of forming the enzyme in a nutrient culture medium, and recovering the formed enzyme from the resultant culture.

The sixth object of the present invention is attained by a method for converting non-reducing saccharides containing a step of allowing the recombinant enzyme to act on non-reducing saccharides, having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, to release trehalose from the saccharides.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

FIG. **1** shows the optimum temperature of an enzyme derived from *Rhizobium* sp. M-11.

FIG. **2** shows the optimum temperature of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. **3** shows the optimum pH of an enzyme derived from *Rhizobium* sp. M-11.

FIG. **4** shows the optimum pH of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. **5** shows the thermal stability of an enzyme derived from *Rhizobium* sp. M-11.

FIG. **6** shows the thermal stability of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. **7** shows the pH stability of an enzyme derived from *Rhizobium* sp. M-11.

FIG. **8** shows the pH stability of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. **9** shows the restriction map of the recombinant DNA pBMU27 according to the present invention. In the figure, the bold-lined part is a DNA encoding an enzyme derived from *Rhizobium* sp. M-11.

FIG. **10** shows the restriction map of the recombinant DNA pBRT32 according to the present invention. In the figure, the bold-lined part is a DNA encoding an enzyme derived from *Arthrobacter* sp. Q36.

DETAILED DESCRIPTION OF THE INVENTION

The DNA according to the present invention exerts the production of the enzyme encoded by the DNA in a manner that the DNA is inserted into an appropriate self-replicable vector to form a replicable recombinant DNA, followed by introducing the recombinant DNA into a host, incapable of producing the enzyme per se but readily replicable, to form a transformant.

Although the recombinant DNA per se does not produce the enzyme, the production of the enzyme encoded by the DNA is attained by introducing the recombinant DNA into a host, incapable of producing the enzyme but replicable with a relative easiness, to form a transformant, and culturing the transformant to produce the enzyme.

The transformant according to the present invention produces the enzyme when cultured.

The recombinant enzyme according to the present invention releases trehalose when acts on non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The recombinant enzyme is readily obtained in a desired amount by culturing the transformant according to the invention.

Non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher are converted into trehalose and glucose and/or maltooligosaccharides.

The present invention is based on the finding of a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Such an enzyme can be obtained from cultures of microorganisms of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36, and the present inventors isolated the enzyme by the combination use of conventional purification methods using column chromatography mainly, examined the properties and features, and revealed the reality, i.e. it is a polypeptide having the following physicochemical properties:

(1) Action Releasing trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher;

(2) Molecular weight About 57,000–68,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE);

(3) Isoelectric point About 3.3–4.6 on isoelectrophoresis;

(4) Optimum temperature Exhibiting an optimum temperature of around 35°–45° C. when incubated at pH 7.0 for 30 min;

(5) Optimum pH Exhibiting an optimum pH of around 6.0–7.5 when incubated at 40° C. for 30 min;

(6) Thermal stability Stable up to a temperature of around 30°–45° C. when incubated at pH 7.0 for 60 min; and

(7) pH Stability Stable up to a pH of around 5.5–10.0 when incubated at 25° C. for 16 hours.

Experiments, which were conducted to reveal the physicochemical properties of the enzymes produced by microorganisms of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36 (the enzymes from *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36 are respectively designated as "enzyme M-11" and "enzyme Q36" hereinafter), are explained in the below:

Experiment 1

Purification of enzyme

Experiment 1-1

Purification of enzyme M-11

In 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid culture medium (pH 7.0) containing 2.0 w/v % "PINE-DEX #4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, Japan, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate, and 0.1 w/v % potassium dihydrogen phosphate, and the flasks were autoclaved at 120° C. for 20 min to effect sterilization. After cooling the flasks a seed culture of *Rhizobium* sp. M-11 was inoculated into each liquid culture medium in each flask, followed by the incubation at 27° C. for 24 hours under rotary-shaking conditions. Twenty L of a fresh preparation of the same liquid culture medium was put in a 30-L jar fermentor and sterilized, followed by inoculating one v/v % of the culture obtained in the above into the sterilized liquid culture medium in the jar fermentor, and incubating it at a pH of 6–8 and 30° C. for 24 hours under aeration-agitation conditions.

Thereafter, about 18 L of the resultant culture was subjected to an ultra-high pressure cell disrupting apparatus to disrupt cells. The resultant suspension was centrifuged to obtain a supernatant, and to about 16 L of which was added ammonium sulfate to give a 20 w/v % saturation, followed by the standing at 4° C. for one hour and the centrifugation to remove sediment. To the resultant supernatant was added ammonium sulfate to give a 60 w/v % saturation, and the

solution was allowed to stand at 4° C. for 24 hours and centrifuged to collect sediment which was then dissolved in a minimum amount of 10 mM phosphate buffer (pH 7.0). The solution thus obtained was dialyzed against 10 mM phosphate buffer (pH 7.0) for 24 hours, and centrifuged to remove insoluble substances. The resultant supernatant was fed to a column packed with "DEAE-TOYOPEARL®", a product for ion-exchange chromatography commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0), followed by feeding to the column a linear gradient buffer of sodium chloride ranging from 0 M to 0.5 M in 10 mM phosphate buffer (pH 7.0). Fractions containing the objective enzyme were collected from the eluate, pooled, dialyzed for 10 hours against 50 mM phosphate buffer (pH 7.0) containing 2 M ammonium sulfate, and centrifuged to remove insoluble substances. Thereafter, the resultant supernatant was fed to a column, which had been packed with "BUTYL TOYOPEARL®", a gel for hydrophobic column chromatography commercialized by Tosoh Corporation, Tokyo, Japan, and equilibrated with 50 mM phosphate buffer (pH 7.0) containing 2 M ammonium sulfate, followed by feeding to the column a linear gradient buffer of ammonium sulfate ranging from 2 M to 0 mM in 50 mM phosphate buffer (pH 7.0). Fractions containing the objective enzyme were collected from the eluate, pooled, fed to a column packed with "TOYOPEARL® HW-55", a product for gel filtration column chromatography commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 50 mM phosphate buffer (pH 7.0), followed by feeding to the column 50 mM phosphate buffer (pH 7.0) and collecting fractions containing the objective enzyme. The enzyme thus obtained had a specific activity of about 240 units/mg protein, and the yield was about 650 units per L of the culture.

Throughout the specification the enzyme activity is expressed by the value measured on the following assay: Place 4 ml of 50 mM phosphate buffer (pH 7.0) containing 1.25 w/v % maltotriosyltrehalose in a test tube, add one ml of an enzyme solution to the tube, and incubate the resultant solution at 40° C. for 30 min to effect enzymatic reaction. Thereafter, one ml of the reaction mixture is mixed with 2 ml of copper reagent to suspend the enzymatic reaction, followed by assaying the reducing activity by the Somogyi-Nelson's method. As a control, an enzyme, which has been previously inactivated by heating at 100° C. for 10 min, is similarly treated as above. One unit activity of the enzyme is defined as the amount of enzyme which increases the reducing power corresponding to one μmol glucose per min under the above conditions.

Experiment 1-2

Purification of enzyme Q36

Similarly as in Experiment 1-1, a seed culture of *Arthrobacter* sp. Q36 was cultured, and the resultant culture was treated to obtain a purified enzyme Q36 having a specific activity of about 450 units/mg protein in a yield of about 650 units per L of the culture.

Experiment 2

Physicochemical property of enzyme

Experiment 2-1

Action

According to the method disclosed in Japanese Patent Application No.349,216/93, a non-reducing saccharide containing 98 w/w % or higher, d.s.b., α-glucosyltrehalose, α-maltosyltrehalose, α-maltotriosyltrehalose, α-maltotetraosyltrehalose or α-maltopentaosyltrehalose. Either of the non-reducing saccharides as a substrate was dissolved in 50 mM phosphate buffer (pH 7.0) into a 20 w/v % solution which was then mixed with 2 units/g substrate of the purified enzyme M-11. or Q36 in Experiment 1 and subjected to an enzymatic reaction at 40° C. for 48 hours. The reaction mixture was desalted in usual manner, fed to "WB-T-330", a column for high-performance liquid chromatography (HPLC) commercialized by Wako Pure Chemical Industries, Ltd., Tokyo, Japan, followed by feeding to the column distilled water at a flow rate of 0.5 ml/min at ambient temperature to isolate saccharides contained in the reaction mixture while monitoring the saccharide concentration of the eluate with "MODEL RI-8012", a differential refractometer commercialized by Tosoh Corporation, Tokyo, Japan. As a control, an aqueous solution which contains either maltotriose, maltotetraose, maltopentaose, maltohexaose or maltoheptaose was similarly treated as above, and the resultant mixture was analyzed. The saccharide compositions of the reaction mixtures were tabulated in Tables 1 and 2.

TABLE 1

Substrate	Saccharide in reaction mixture	Saccharide composition (%)
α-Glucosyltrehalose	Trehalose	17.5
	Glucose	6.5
	α-Glucosyltrehalose	76.0
α-Maltosyltrehalose	Trehalose	44.3
	Maltose	44.4
	α-Maltosyltrehalose	11.3
α-Maltotriosyltrehalose	Trehalose	39.5
	Maltotriose	60.0
	α-Maltotriosyltrehalose	0.5
α-Maltotetraosyltrehalose	Trehalose	34.2
	Maltotetraose	65.5
	α-Maltotetraosyltrehalose	0.3
α-Maltopentaosyltrehalose	Trehalose	29.1
	Maltopentaose	70.6
	α-Maltopentaosyltrehalose	0.3
Maltotriose	Maltotriose	100.0
Maltotetraose	Maltotetraose	100.0
Maltopentaose	Maltopentaose	100.0
Maltohexaose	Maltohexaose	100.0
Maltoheptaose	Maltoheptaose	100.0

TABLE 2

Substrate	Saccharide in reaction mixture	Saccharide composition (%)
α-Glucosyltrehalose	Trehalose	19.3
	Glucose	10.2
	α-Glucosyltrehalose	70.5
α-Maltosyltrehalose	Trehalose	44.5
	Maltose	44.4
	α-Maltosyltrehalose	11.1
α-Maltotriosyltrehalose	Trehalose	38.8
	Maltotriose	60.7
	α-Maltotriosyltrehalose	0.5
α-Maltotetraosyltrehalose	Trehalose	34.1
	Maltotetraose	65.7
	α-Maltotetraosyltrehalose	0.2
α-Maltopentaosyltrehalose	Trehalose	29.3
	Maltopentaose	70.4
	α-Maltopentaosyltrehalose	0.3
Maltotriose	Maltotriose	100.0
Maltotetraose	Maltotetraose	100.0
Maltopentaose	Maltopentaose	100.0
Maltohexaose	Maltohexaose	100.0
Maltoheptaose	Maltoheptaose	100.0

As shown in Tables 1 and 2, enzymes M-11 and Q36 almost quantitatively released trehalose, glucose and maltooligosaccharides from non-reducing saccharides having a

trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. These enzymes did not act on maltooligosaccharides, as a substrate, having a degree of glucose polymerization of 3 or higher. These facts indicate that these enzymes selectively act on non-reducing saccharides having a trehalose structure as an end unit and having a degree of polymerization degree of 3 or higher, and specifically hydrolyze the glycosidic bond between trehalose- and glycosyl-residues. Such an enzyme has never been reported and is estimated to have a novel enzymatic reaction mechanism.

Experiment 2-2

Molecular weight

In accordance with the method reported by U. K. Laemmli in *Nature*, Vol.227, pp.680–685 (1970), the purified enzymes M-11 and Q36 in Experiment 1 were respectively electrophoresed on sodium dodecyl sulfate polyacrylamide gel electrophoresis to show a single protein band at a position corresponding to about 57,000–68,000 daltons. The marker proteins used in this experiment were myosin (MW=200,000 daltons), β-galactosidase (MW=116,250 daltons), phosphorylase B (MW=97,400 daltons), serum albumin (MW=66,200 daltons) and ovalbumin (MW=45,000 daltons).

Experiment 2-3

Isoelectric point

The purified enzymes M-11 and Q36 obtained in Experiment 1 gave an isoelectric point of about 3.3–4.6 on isoelectrophoresis.

Experiment 2-4

Optimum temperature

The optimum temperature of the purified enzymes M-11 and Q36 obtained in Experiment 1 was about 35°–45° C. as shown in FIGS. 1 and 2 when incubated in usual manner in 50 mM phosphate buffer (pH 7.0) for 30 min.

Experiment 2-5

Optimum pH

The optimum pH of the purified enzymes M-11 and Q36 obtained in Experiment 1 was about 6.0–7.5 as shown in FIGS. **3** and 4 when experimented in usual manner by incubating them at 40° C. for 30 min in 50 mM acetate buffer, phosphate buffer or sodium carbonate-sodium hydrogen carbonate buffer having different pHs.

Experiment 2-6

Thermal stability

The purified enzymes M-11 and Q36 obtained in Experiment 1 were stable up to a temperature of about 30°–45° C. as shown in FIGS. **5** and **6** when experimented in usual manner by incubating them in 50 mM phosphate buffer (pH 7.0) for 60 min.

Experiment 2-7

pH Stability

The purified enzymes M-11 and Q36 obtained in Experiment 1 were stable up to a pH of about 5.5–10.0 as shown in FIGS. **7** and **8** when experimented in usual manner by incubating them at 25° C. for 16 hours in 50 mM acetate buffer, phosphate buffer or sodium carbonate-sodium hydrogen carbonate buffer having different pHs.

Experiment 2-8

Amino acid sequence containing the N-terminal

The amino acid sequence containing the N-terminal of the purified enzyme M-11 obtained in Experiment 1 was analyzed on "MODEL 470A", a gas-phase protein sequencer commercialized by Applied Biosystems, Inc., Foster City, USA, to reveal that it has the amino acid sequence as shown in SEQ ID NO: 5.

The amino acid sequence containing the N-terminal of the purified enzyme Q36 was analyzed similarly as above to reveal that it has the amino acid sequence as shown in SEQ ID NO: 6.

Experiment 2-9

Partial amino acid sequence

An adequate amount of the purified enzyme M-11 obtained in Experiment 1-1 was weighed, dialyzed against 10 mM Tris-HCl buffer (pH 9.0) at 4° C. for 18 hours, and admixed with 10 mM Tris-HCl buffer (pH 9.0) to give a concentration of about one mg/ml of the enzyme. About one ml of the resultant solution was placed in a container, admixed with 10 μg lysyl endopeptidase, and incubated at 30° C. for 22 hours to partially hydrolyze the enzyme. The resultant hydrolysate was applied to "CAPCELL-PAK C18", a column for reverse-phase high-performance liquid chromatography commercialized by Shiseido Co., Ltd., Tokyo, Japan, which had been previously equilibrated with 0.1 v/v % trifluoroacetate containing 16 v/v % aqueous acetonitrile, followed by feeding to the column 0.1 v/v % trifluoroacetate at a flow rate of 0.9 ml/min while increasing the concentration of acetonitrile from 16 v/v % to 64 v/v % to separately collect fractions containing a peptide fragment eluted about 43 min or about 57 min after the initiation of feeding (the peptide fragments were respectively named "peptide fragment A" and "peptide fragment B"). Fractions containing the peptide fragment A or B were separately pooled, dried in vacuo, and dissolved in 0.1 v/v % trifluoroacetate containing 50 v/v % aqueous acetonitrile. Similarly as in Experiment 2-8, the peptide fragments A and B were analyzed to reveal that they have the amino acid sequences as shown in SEQ ID NOs: 7 and 8, respectively.

Similarly as in enzyme M-11, enzyme Q36 obtained in Experiment 1-2 was partially hydrolyzed, and the resultant was fed to "μBONDAPAK C18", a column for reverse-phase high-performance liquid chromatography commercialized by Japan Millipore Ltd., Tokyo, Japan, which had been previously equilibrated with 0.1 v/v % trifluoroacetate containing 24 v/v % aqueous acetonitrile, followed by feeding to the column 0.1 v/v % trifluoroacetate containing 24 v/v % aqueous acetonitrile while increasing the concentration of aqueous acetonitrile from 24 v/v % to 44 v/v % at a flow rate of 0.9 ml/ml. Fractions containing a peptide fragment eluted about 4 min or about 24 min after the initiation of feeding (the fractions were respectively called "peptide fragment C" and "peptide fragment D" hereinafter) were respectively collected, pooled, dried in vacuo, and dissolved in 0.1 v/v % trifluoroacetate containing 50 v/v % aqueous acetonitrile. Analyses of the peptide fragments C and D conducted similarly as above have revealed that they have amino acid sequences as shown in SEQ ID NOs: 9 10 respectively.

No enzyme having these physicochemical properties has been known, and this concluded that it is a novel substance. Referring to *Rhizobium* sp. M-11 , it is a microorganism which was isolated from a soil of Okayama-city, Okayama, Japan, deposited on Dec. 24, 1992, in National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology, Tsukuba, Ibaraki, Japan, and accepted under the accession number of FERM BP-4130, and it has been maintained by the institute. *Arthrobacter* sp. Q36 is a microorganism which was isolated from a soil of Soja-city, Okayama, Japan, deposited on Jun. 3, 1993, in the same institute, and accepted under the accession number of FERM BP-4316, and it has been maintained by the institute. Japanese Patent Application No.340,343/93, applied by the same applicant, discloses the properties and features of the non-reducing saccharide-forming enzyme as well as the detailed bacteriological properties of these microorganisms.

The present inventors energetically screened the chromosomal DNA of *Rhizobium* sp. M-11 by using an oligonucle-

otide as a probe which had been chemically synthesized based on the partial amino acid sequence of enzyme M-11 as revealed in Experiment 2-8 or 2-9, and obtained a DNA fragment which consists of 1,767 base pairs having the base sequence as shown in the following SEQ ID NO: 1 that initiates from the 5'-terminus. The decoding of the base sequence of the enzyme has revealed that it has an amino acid sequence consisting of 589 amino acids as shown in SEQ ID NO: 2.

Similarly as in enzyme M-11, the chromosomal DNA of enzyme Q36 was screened by using an oligonucleotide as a probe which had been chemically synthesized based on a partial amino acid sequence of enzyme Q36, and this yielded a DNA fragment having a base sequence consisting of 1,791 base pairs as shown in SEQ ID NO: 3. The base sequence was decoded to reveal that enzyme Q36 has an amino acid sequence consisting of 597 amino acids as shown in SEQ ID NO: 4.

The sequential experimental steps used for revealing the base sequence and amino acid sequence as shown in SEQ ID NOs: 1 to 4 are summarized as below:

(1) The enzyme was isolated from a culture of a donor microorganism and highly purified. The purified enzyme was partially hydrolyzed with protease, and the resultant 2 different types of peptide fragments were isolated and determined their amino acid sequences;

(2) Separately, a chromosomal DNA was isolated from a donor microorganism's cell, purified and partially digested by a restriction enzyme to obtain a DNA fragment consisting of about 2,000–6,000 base pairs. The DNA fragment was ligated by DNA ligase to a plasmid vector, which had been previously cut with a restriction enzyme, to obtain a recombinant DNA;

(3) The recombinant DNA was introduced into *Escherichia coli* to obtain transformants, and from which an objective transformant containing a DNA encoding the enzyme was selected by the colony hybridization method using an oligonucleotide, as a probe, which had been chemically synthesized based on the aforesaid partial amino acid sequence; and

(4) The recombinant DNA was obtained from the selected transformant and annealed with a primer, followed by allowing a DNA polymerase to act on the resultant to extend the primer, and determining the base sequence of the resultant complementary chain DNA by the dideoxy chain termination method. The comparison of an amino acid sequence, estimable from the determined base sequence with the aforesaid amino acid sequence, confirmed that the base sequence encodes the enzyme.

The recombinant enzyme as referred to in the specification mean the whole recombinant enzymes which are preparable by the recombinant DNA technology and capable of releasing trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Generally, the recombinant enzyme according to the present invention has a revealed amino acid sequence, and, as an example, the amino acid sequence as shown in SEQ ID NO: 2 or 4 which initiates from the N-terminal, as well as homologous ones to it, can be mentioned. Variants having amino acid sequences homologous to the one as shown in SEQ ID NO: 2 or 4 can be obtained by replacing one or more amino acids in SEQ ID NO: 2 or 4 with other amino acids without substantially altering the inherent activity of the enzyme. Although even when used the same DNA and it also depends on hosts into which the DNA is introduced, as well as on ingredients and components of nutrient culture media used for culturing transformants, and their cultivation temperature and pH, there may be produced modified enzymes which have amino acid sequences similar to that of SEQ ID NO: 2 or 4, as well as having the enzymatic activity inherent to the enzyme encoded by the DNA but defective one or more amino acids located near to the N-terminal of the amino acid sequence of SEQ ID NO: 2 or 4 and/or having one or more amino acids newly added to the N-terminal by the modification of intracellular enzymes of host s after the DNA expression. In view of the technical background in the art, the enzyme as referred to in the present invention includes those which have the amino acid sequence corresponding to that of SEQ ID NO: 2 or 4, and those which substantially have the amino acid sequence as shown in SEQ ID NO: 2 or 4 except that one or more amino acids in the amino acid sequence are defected, newly added to or replaced with other amino acids, as long as they release trehalose form non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

In this field, it is known that one or more bases in DNAs can be replaced with other bases by the degeneracy of genetic code without altering the amino acid sequences encoded by the DNAs. Based on this the DNA according to the present invention includes DNAs which contain the base sequence of SEQ ID NO: 1 or 3 and other DNAs, wherein one or more bases are replaced with other bases by degeneracy of genetic code, as long as they encode enzymes having the amino acid sequence as shown in SEQ ID NO: 2 or 4 and homologous variants thereof.

According to the today's recombinant DNA technology, the determination of base sequences from the 5'-termini of DNAs define their complementary base sequences. Therefore, the DNA according to the present invention also includes complementary base sequences corresponding to any one of the aforesaid base sequences. Needless to say, one or more bases in the base sequence, which encodes the enzyme or their variants, can be readily replaced with other bases to allow the DNA to actually express the enzyme production in hosts.

The DNA according to the present invention is as described above, and any DNA derived from natural resources and those artificially synthesized can be used in the present invention as long as they have the aforementioned base sequences. The natural resources of the DNA according to the present invention are, for example, microorganisms of the genera *Rhizobium, Arthrobacter, Brevibacterium* and *Micrococcus*, i.e. *Rhizobium* sp. M-11 (FERM BP-4130), *Arthrobacter* sp. Q36 (FERM BP-4316), *Brevibacterium helovolum* (ATCC 11822) and *Micrococcus roseus* (ATCC 186) from which genes containing the present DNA can be obtained. These microorganisms can be inoculated in nutrient culture media and cultured for about 1–3 days under aerobic conditions, and the resultant cells were collected from the cultures and subjected to ultrasonication or treated with a cell-wall lysis enzyme such as lysozyme or β-glucanase to extract genes containing the present DNA. In this case, a proteolytic enzyme such as protease can be used along with the cell-wall lysis enzyme, and, in the case of treating the cells with ultrasonication, they may be treated in the presence of a surfactant such as sodium dodecyl sulfate (SDS) or treated with freezing- and thawing-methods. The objective DNA is obtainable by treating the resultant with phenol extraction, alcohol sedimentation, centrifugation, protease treatment and/or ribonuclease treatment used in general in the art.

To artificially synthesize the DNA according to the present invention, it can be chemically synthesized by using

the base sequence as shown in SEQ ID NO: 1 or 3, or can be obtained in plasmid form by inserting a DNA, which encodes the amino acid sequence as shown in SEQ ID NO: 2 or 4, into an appropriate self-replicable vector to obtain a recombinant DNA, introducing the recombinant DNA into an appropriate host to obtain a transformant, culturing the transformant, separating the proliferated cells from the resultant culture, and collecting plasmids containing the DNA from the cells.

The present invention further relates to replicable recombinant DNAs which express the production of the enzyme according to the invention when introduced into microorganisms as well as plant- and animal-cells which do not produce the enzyme inherently but are readily proliferative. Such a recombinant DNA, which generally contains the aforesaid DNA and a self-replicable vector, can be prepared by conventional method with a relative easiness when the material DNA is in hand. Examples of such a vector are plasmid vectors such as pBR322, pUC18, Bluescript II SK(+), pUB110, pTZ4, pC194, pHV14, TRp7, TEp7, pBS7, etc.; and phage vectors such as λgt·λC, λgt·λB, ρ11, ϕ1, ϕ105,etc. Among these plasmid- and phage-vectors, pBR322, pUC18, Bluescript II SK(+), λgt·λC and λgt·λB are satisfactorily used in case that the present DNA should be expressed in *Escherichia coli*, while pUB110, pTZ4, pC194, ρ11, ϕ1 and ϕ105 are satisfactorily used to express the DNA in microorganisms of the genus *Bacillus*. The plasmid vectors pHV14, TRp7, TEp7 and pBS7 are suitably used when the recombinant DNA is allowed to grow in 2 or more hosts.

The methods used to insert the present DNA into such vectors in the present invention may be conventional ones generally used in this field. A gene containing the present DNA and a self-replicable vector are first digested by a restriction enzyme and/or ultrasonic disintegrator, then the resultant DNA fragments and vector fragments are ligated. To digest DNAs and vectors, restriction enzymes which specifically act on nucleotides, particularly, type II restriction enzymes, more particularly, Sau 3AI, Eco RI, Hind III, Bam HI, Sal I, Xba I, Sac I, Pst I, etc., facilitate the ligation of the DNA fragments and vector fragments. The ligation of the DNA fragments and vector fragments is effected by annealing them first if necessary, then subjected to the action of a DNA ligase in vivo or in vitro. The recombinant DNA thus obtained is replicable without substantial limitation by introducing it into appropriate hosts, and culturing the resultant transformants.

The recombinant DNA according to the present invention can be introduced into appropriate host microorganisms including *Escherichia coli* and those of the genus *Bacillus* as well as actinomyces and yeasts. In the case of using *Escherichia coli* as a host, it can be cultured in the presence of the recombinant DNA and calcium ion, while in the case of using the microorganisms of the genus *Bacillus* the competent cell method and the colony hybridization method can be employed. Desired transformants can be cloned by the colony hybridization method or by culturing a variety of transformants in nutrient culture media containing non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, and selecting the objective transformants which release trehalose form the non-reducing saccharides.

The transformants thus obtained extracellularly produce the objective enzyme when cultured in nutrient culture media. Generally, liquid media in general supplemented with carbon sources, nitrogen sources and minerals, and, if necessary, further supplemented with a small amount of amino acids and vitamins can be used as the nutrient culture media. Examples of the carbon sources are saccharides such as starch, starch hydrolysate, glucose, fructose and sucrose. Examples of the nitrogen sources are organic- and inorganic-substances containing nitrogen such as ammonia, ammonium salts, urea, nitrate, peptone, yeast extract, defatted soy been, corn steep liquor and beef extract. Cultures containing the objective enzyme can be prepared by inoculating the transformants into nutrient culture media, and incubating them at a temperature of 25°–65° C. and a pH of 2–8 for about 1–6 days under aerobic aeration-agitation conditions. Such a culture can be used intact as an enzyme preparation, and, usually, it may be disrupted with ultrasonic disintegrator and/or cell-wall lysis enzymes prior to use, followed by separating the enzyme from the intact cells and cell debris by filtration and/or centrifugation, and purifying the enzyme. The methods used for purifying the enzyme in the invention include conventional ones in general. From cultures the intact cells and cell debris are eliminated and subjected to one or more methods such as concentration, salting out, dialysis, separately sedimentation, gel filtration chromatography, ion exchange chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectric point electrophoresis.

As is described above, the enzyme exerts a distinct activity of forming trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, and such an activity has not yet been found in any conventional enzymes. Therefore, the use of the enzyme facilitates the preparation of trehalose in a relatively-high yield and efficiency from non-reducing saccharides such as α-glucosyltrehalose, α-maltosyltrehalose, α-maltotriosyltrehalose, α-maltotetraosyltrehalose and α-maltopentaosyltrehalose in a considerably-high yield. These non-reducing saccharides can be obtained in a satisfactorily-high yield from starch hydrolysates, which are obtained by treating amylaceous substances such as starch, amylose and amylopectin prepared with acids and/or amylases, by using non-reducing saccharide-forming enzyme as disclosed in Japanese Patent Application No.349,216/93. Thus, trehalose, whose industrial preparation has been difficult, can be prepared from starch and amylaceous substances as a material with a relative easiness and in a desired amount when the present enzyme and the non-reducing saccharide-forming enzyme, as disclosed in Japanese Patent Application No.349,216/93, are used in combination.

As described in "*Handbook of Amylases and Related Enzymes*", 1st edition, edited by The Amylase Research Society of Japan, published by Pergamon Press plc, Oxford, England (1988), α-amylase, maltotetraose-forming amylase, maltopentaose-forming amylase and maltohexaose-forming amylase are especially useful to prepare the reducing amylaceous saccharides used in the invention, and, the use of any one of these amylases readily yields amylaceous saccharide mixtures rich in reducing amylaceous saccharides having a degree of glucose polymerization of 3 or higher in a considerably-high yield. If necessary, the combination use of such an amylase and a starch debranching enzyme such as pullulanase or isoamylase can increase the yield of the reducing amylaceous saccharides usable as a substrate for the non-reducing saccharide-forming enzyme, i.e. the non-reducing saccharides can be obtained by coexisting the non-reducing saccharide-forming enzyme in an aqueous solution containing as a substrate one or more of the reducing amylaceous saccharides in an amount up to a concentration of 50 w/v %,

and subjecting the solution to an enzymatic reaction at a temperature of about 40°–55° C. and a pH of about 6–8 until a desired amount of the objective non-reducing saccharides are formed.

Usually, in the present conversion method, the recombinant enzyme according to the present invention is allowed to coexist in the aforesaid aqueous solution containing one or more of the non-reducing amylaceous saccharides, and to enzymatically react with the saccharides while keeping at a prescribed temperature and pH until a desired amount of trehalose is released.

Although the enzymatic reaction proceeds even below a concentration of 0.1 w/v % of a substrate, a higher concentration of 2 w/v %, preferably, 5–50 w/v % of a substrate can be satisfactorily used to apply the present conversion method to an industrial-scale production. The temperature and pH used in the enzymatic reaction are set within the ranges of which do not inactivate the recombinant enzyme and allow the recombinant enzyme to effectively act on substrates, i.e. a temperature up to about 55° C., preferably, a temperature in the range of about 40°–55° C., and a pH of 5–10, preferably, a pH in the range of about 6–8. The amount and reaction time of the present recombinant enzyme are chosen dependently on the enzymatic reaction conditions. The enzymatic reaction effectively converts non-reducing saccharides into saccharide compositions containing trehalose and glucose and/or maltooligosaccharides, and, in the case of using α-maltotriosyltrehalose as a substrate, the conversion rate reaches to approximately 100%. In the case of simultaneously subjecting starch hydrolysates to the action of either of the above amylases together with the non-reducing saccharide-forming enzyme and the present recombinant enzyme, non-reducing saccharides are formed from the hydrolysates while hydrolyzed into glucose and/or maltooligosaccharides, and because of this saccharide compositions with a relatively-high trehalose content can be effectively obtained in a relatively-high yield.

The reaction products obtained by the present conversion reaction can be used intact, and, usually, they are purified prior to use: Insoluble substances are eliminated from the reaction products by filtration and centrifugation, and the resultant solutions are decolored with activated charcoal, desalted and purified on ion exchangers, and concentrated into syrupy products. Dependently on their use, the syrupy products are dried in vacuo and spray-dried into solid products. In order to obtain products which substantially consist of non-reducing saccharides, the above mentioned syrupy products are subjected to one or more methods such as chromatography using an ion exchanger, activated charcoal and silica gel to separate saccharides, separately sedimentation using alcohol and/or acetone, membrane filtration, fermentation by yeasts, and removal and decomposition of reducing saccharides by alkalis. The methods to treat a large amount of reaction mixture are, for example, fixed bed- or pseudomoving bed-ion exchange column chromatography as disclosed in Japanese Patent Laid-Open Nos.23,799/83 and 72,598/83, and such a method enables an effective industrial-scale production of products with a relatively-high trehalose content.

These trehalose and compositions containing the same have a wide applicability to a variety of products which are apt to be readily damaged by the reducibility of saccharide sweeteners: For example, they can be satisfactorily used as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant in food products in general, cosmetics and pharmaceuticals.

The following examples explain the present invention in more detail, and the techniques themselves used in the examples are conventional ones in this field, for example, those described by J. Sumbruck et al. in "*Molecular Cloning A Laboratory Manual*", 2nd edition, published by Cold Spring Harbor Laboratory Press (1989).

Example 1

Preparation of recombinant DNA containing DNA encoding enzyme M-11 and transformant

Example 1-1

Preparation of chromosomal DNA

A seed culture of *Rhizobium* sp. M-11 was inoculated into bacto nutrient broth medium (pH 7.0), and cultured at 27° C. for 24 hours with a rotary shaker. The cells were separated from the resultant culture by centrifugation, suspended in TES buffer (pH 8.0), admixed with 0.05 w/v % lysozyme, and incubated at 37° C. for 30 min. The resultant was freezed at −80° C. for one hour, admixed with TSS buffer (pH 9.0), heated to 60° C., and further admixed with a mixture solution of TES buffer and phenol, and the resultant solution was chilled with ice, followed by centrifugally collecting the precipitated crude chromosomal DNA. To the supernatant was added 2 fold volumes of cold ethanol, and the re-precipitated crude chromosomal DNA was collected, suspended in SSC buffer (pH 7.1), admixed with 7.5 μg ribonuclease and 125 μg protease, and incubated at 37° C. for one hour. Thereafter, a mixture solution of chloroform and isoamyl alcohol was added to the reaction mixture to extract the objective chromosomal DNA, and admixed with cold ethanol, followed by collecting the formed sediment containing the chromosomal DNA. The purified chromosomal DNA thus obtained was dissolved in SSC buffer (pH 7.1) to give a concentration of about one mg/ml, and the resultant solution was freezed at −80° C.

Example 1-2

Preparation of recombinant DNA pBMU27 and transformant BMU27

About one ml of the purified chromosomal DNA obtained in Example 1-1 was placed in a container, admixed with about 35 units of Sau 3AI, a restriction enzyme, and enzymatically reacted at 37° C. for about 20 min to partially digest the chromosomal DNA, followed by recovering a DNA fragment consisting of about 2,000–6,000 base pairs by means of sucrose density-gradient ultracentrifugation. One μg of Bluescript II SK(+), a plasmid vector, was provided, subjected to the action of Bam HI, a restriction enzyme, to completely digest the plasmid vector, admixed with 10 μg of the DNA fragment and 2 units of T4 DNA ligase, and allowed to stand at 4° C. overnight to ligate the DNA fragment to the vector fragment. To the resultant recombinant DNA was added 30 μl of "Epicurian Coli® XLI-Blue", competent cell commercialized by Toyobo Co., Ltd., Tokyo, Japan, allowed to stand under ice-chilling conditions for 30 min, heated to 42° C., admixed with SOC broth, and incubated at 37° C. for one hour to introduce the recombinant DNA into *Escherichia coil.*

The resultant transformant was inoculated into agar plate (pH 7.0) containing 50 μg/ml of 5-bromo-4-chloro-3-indolyl-β-galactoside, and cultured at 37° C. for 18 hours, followed by placing a nylon film on the agar plate to fix thereon about 6,000 colonies formed on the agar plate. Based on the amino acid sequence located at positions from 8 to 13 as shown in SEQ ID No: 7, i.e. Phe-Asp-Ile-Trp-Ala-Pro, the base sequence of probe 1 represented by 5'-TTYGAYATHTGGGCNCC-3' (SEQ ID NO: 15) was chemically synthesized, labelled with ^{32}P, and hybridized with the colonies of transformants fixed on the nylon film, followed by selecting 14 transformants which exhibited a strong hybridization.

The objective recombinant DNA was selected in usual manner from the 14 transformants, and, in accordance with the method described by E. M. Southern in *Journal of Molecular Biology*, Vol.98, pp.503–517 (1975), the recombinant DNA was hybridized with probe 2 having the base sequence as shown in SEQ ID NO: 8, which had been chemically synthesized based on the amino acid sequence located at positions from 2 to 6, i.e. Asp-Trp-Ala-Glu-Ala, in SEQ ID NO: 8, followed by selecting a recombinant DNA strongly hybridized with the probe 2. The recombinant DNA and transformant thus selected were respectively named "pBMU27" and "BMU27".

The transformant BMU27 was inoculated into L-broth (pH 7.0) containing 100 μg/ml ampicillin, and cultured at 37° C. for 24 hours by a rotary shaker. After completion of the culture, the resultant cells were collected from the culture by centrifugation, and treated with the alkaline method in general to extracellularly extract a recombinant DNA. The extract was in usual manner purified and analyzed to reveal that the recombinant DNA pBMU27 consists of about 5,700 base pairs and has the structure expressed by the restriction map as shown in FIG. **9**. It was found that, as shown in FIG. **9**, the DNA which consists of 1,767 base pairs for encoding the enzyme M-11 is positioned in the downstream near to the digested site of Eco RV, a restriction enzyme.

Example 1-3

Production of enzyme by transformant BMU27

A liquid nutrient culture medium consisting of 2.0 w/v % "PINE-DEX #4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, Japan, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.I w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate was adjusted to pH 7.0, admixed with 50 μg/ml ampicillin, autoclaved at 120° C. for 20 min, cooled and inoculated with a seed culture of transformant BMU27 obtained in Example 1-2, followed by culturing the transformant at 37° C. for 24 hours by a rotary shaker. The resultant culture was treated with ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the enzyme activity to find that one L of the culture yielded about 4,000 units of the enzyme.

As a control, a seed culture of *Escherichia coli* XLI-Blue or *Rhizobium* sp. M-11 was inoculated in the same fresh preparation of the same liquid nutrient culture medium but free of ampicillin, and, in the case of culturing *Rhizobium* sp. M-11, it was cultured and treated similarly as above except that the cultivation temperature was set to 30° C. Assaying the resultant activity, one L culture of *Rhizobium* sp. M-11 yielded about 2,000 units of the enzyme, and the yield was significantly lower than that of transformant BMU27. *Escherichia coli* XLI-Blue used as a host did not form the enzyme.

Thereafter, the enzyme produced by the transformant MBU27 was purified similarly as in Experiment 1-1, and examined on the properties and characters. As a result, it was revealed that it has substantially the same physicochemical properties as enzyme M-11, i.e. it has a molecular weight of about 57,000–68,000 daltons on SDS-PAGE and an isoelectric point of about 3.3–4.6 on isoelectrophoresis. The results indicate that the present enzyme can be prepared by the recombinant DNA technology, and the yield can be significantly increased thereby.

Example 2

Preparation of complementary chain DNA derived from *Rhizobium* sp. M-11, and determination for its base sequence and amino acid sequence

Two μg of the recombinant DNA pBMU27 obtained in Example 1-2 was provided, admixed with 2 M aqueous sodium hydroxide solution to effect degeneration, and admixed with an adequate amount of cold ethanol, followed by collecting the formed sediment containing a template DNA and drying the sediment in vacuo. To the template DNA were added 50 pmole/ml of a chemically synthesized primer 1 represented by 5'-GTAAAACGACGGCCAGT-3' (SEQ ID NO: 16), 10 μl of 40 mM Tris-HCl buffer (pH 7.5) containing 20 mM magnesium chloride and 20 mM sodium chloride, and the mixture was incubated at 65° C. for 2 min to effect annealing and admixed with 2 μl of an aqueous solution containing dATP, dGTP and dTTP in respective amounts of 7.5 μM, 0.5 μl of [α-^{32}P]dCTP (2 mCi/ml), one μl of 0.1 M dithiothreitol, and 2 μl of 1.5 units/ml T7 DNA polymerase, followed by incubating the resultant mixture at 25° C. for 5 min to extend the primer 1 from the 5'-terminus to the 3'-terminus. Thus, a complementary chain DNA was formed.

The reaction product containing the complementary chain DNA was divided into quarters, to each of which 2.5 μl of 50 mM aqueous sodium chloride solution containing 80 μM dNTP and 8 μM ddATP, ddCTP, ddGTP or ddTTP was added, and the resultant mixture was incubated at 37° C. for 5 min, followed by suspending the reaction by the addition of 4 μl of 98 v/v % aqueous formamide solution containing 20 mM EDTA, 0.05 w/v % bromophenol blue, and 0.05 w/v % xylene cyanol. The reaction mixture was heated with a boiling-water bath for 3 min, and a portion of which was placed on a gel containing 6 w/v % polyacrylamide, and electrophoresed by energizing the gel with a constant voltage of about 2,000 volts to separate DNA fragments, followed by fixing the gel in usual manner, drying the gel and subjecting the resultant gel to autoradiography.

Analyses of the DNA fragments separated on the radiogram revealed that the complementary chain DNA contains the base sequence consisting of about 2,161 base pairs as shown in SEQ ID NO: 11. An amino acid sequence estimable from the base sequence was as shown in SEQ ID NO: 12 and was compared with the amino acid sequence containing the N-terminal or the partial amino acid sequence of enzyme M-11 as shown in SEQ ID NO: 5, 7 or 8. As a result, it was found that the amino acid sequence containing the N-terminal of SEQ ID NO: 5 corresponds to the amino acid sequence located at positions from 8 to 27 in SEQ ID NO: 12, and the partial amino acid sequence of SEQ ID NO: 7 or 8 corresponds to the amino acid sequence located at positions from 10 to 30 or at positions from 493 to 509 in SEQ ID NO: 12. These results indicate that enzyme M-11 has the amino acid sequence of SEQ ID NO: 2, and it is encoded by the DNA having the base sequence as shown in SEQ ID NO: 1.

Example 3

Preparation of recombinant DNA, containing DNA derived from *Arthrobacter* sp. Q36, and transformant

Example 3-1

Preparation of chromosomal DNA

Similarly as in Example 1-1, a chromosomal DNA was isolated from *Arthrobacter* sp. Q36, purified and dissolved in SSC buffer (pH 7.1) to give a concentration of about one mg/ml, and the resultant solution was freezed at −80° C. for storage.

Example 3-2

Preparation of recombinant DNA pBRT32 and transformant BRT32

The purified chromosomal DNA obtained in Example 3-1 was partially digested similarly as in Example 1-2, followed

by recovering a DNA fragment consisting of about 2,000–6,000 base pairs by sucrose density gradient ultracentrifugation. The DNA fragment was ligated to a lysate of Bluescript II SK(+) which had been treated with Bam HI, and the resultant recombinant DNA was introduced into *Escherichia coli* XLI-Blue. The transformants thus obtained were cultured similarly as in Example 1-2 on agar plates containing 5-bromo-4-chloro-3-indolyl-β-galactoside, and the formed about 5,000 colonies were fixed on a nylon film, while the probe 3 represented by 5'-ATGGGNTGGGAYCCNGC-3' (SEQ ID NO: 17) was chemically synthesized based on the amino acid sequence of Met-Gly-Trp-Asp-Pro-Ala located at positions from 5 to 10 in SEQ ID NO: 9, labelled with ^{32}P, and hybridized with transformant colonies which had been fixed on the nylon film, followed by selecting 10 transformants which strongly hybridized with the probe 3.

Similarly as in Example 1-2, the objective recombinant DNA was selected from 10 transformants, and hybridized with probe 4 represented by 5'-TAYGAYGTNTGGGC-3' (SEQ ID NO: 18) which had been chemically synthesized based on the amino acid sequence of Tyr-Asp-Val-Trp-Ala located at positions from 8 to 12 in SEQ ID NO: 10, followed by selecting a recombinant DNA which strongly hybridized with probe 4. The recombinant DNA and transformant thus selected were respectively named "pBRT32" and "BRT32".

The transformant BRT32 was inoculated into L-broth containing ampicillin, and cultured similarly as in Example 1-2, and the proliferated cells were collected from the resultant culture, and from which a recombinant DNA was extracted, purified and analyzed to reveal that the recombinant DNA pBRT32 consists of about 6,200 base pairs and has the structure of the restriction map as shown in FIG. **10**. As shown in FIG. **10**, it was revealed that the DNA, which consists of 1,791 base pairs for encoding the DNA of enzyme Q36, is located in the downstream near to the cleavage site of Kpn I.

Example 3-3

Production of enzyme by transformant BRT32

A liquid nutrient culture medium consisting of 2.0 w/v % "PINE-DEX #4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate was adjusted to pH 7.0, admixed with 50 μg/ml ampicillin, autoclaved at 120° C. for 20 min, cooled and inoculated with a seed culture of the transformant BRT32 obtained in Example 3-2, followed by culturing the transformant at 37° C. for 24 hours by a rotary shaker. The resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the present enzyme activity to find that one L of the culture yielded about 3,900 units of the enzyme.

As a control, a seed culture of *Escherichia coli* XLI-Blue or *Arthrobacter* sp. Q36 was inoculated into a fresh preparation of the same liquid nutrient culture medium but free of ampicillin, and, in the case of culturing *Arthrobacter* sp. Q36, it was cultured and treated similarly as above except that the cultivation temperature was set to 30° C. Assaying the enzyme activity, one L of the culture of *Arthrobacter* sp. Q36 yielded about 1,800 units of the enzyme, and the yield was significantly lower than that of the transformant BRT32. The *Escherichia coli* XLI-Blue used as a host did not form the enzyme.

Thereafter, the enzyme produced by the transformant BRT32 was purified similarly as in Experiment 1-1, and examined on the properties and characters to reveal that it has substantially the same physicochemical properties as that of enzyme Q36, i.e. it has a molecular weight of about 57,000–68,000 daltons on SDS-PAGE and an isoelectric point of about 3.3–4.6 on isoelectrophoresis. These results indicate that the enzyme can be prepared by the recombinant DNA technology, and the yield can be significantly increased thereby.

Example 4

Preparation of complementary chain DNA derived from *Arthrobacter* sp. Q36, and determination for its base sequence and amino acid sequence

The recombinant DNA pBRT32 obtained in Example 3-2 was similarly treated as in Example 2 to form a template DNA which was then annealed together with the primer 1, followed by allowing T7 DNA polymerase to act on the resultant to extend the primer 1 from the 5'-terminus to the 3'-terminus to obtain a complementary chain DNA. Similarly as in Example 2, the complementary chain DNA was subjected to the dideoxy chain terminator method to analyze DNA fragments which had been isolated on a radiogram. The result revealed that the complementary chain DNA contained a base sequence consisting of 2,056 base pairs as shown in SEQ ID NO: 13. An amino acid sequence estimable from the base sequence was as shown in SEQ ID NO: 14, and compared with the amino acid sequence containing the N-terminal or the partial amino acid sequence of SEQ ID NO: 6, 9 or 10. As a result, it was found that the amino acid sequence of SEQ ID NO: 6 corresponds to that located at positions from 2 to 21 in SEQ ID NO: 14, and that the partial amino acid sequence in SEQ ID NO: 9 or 10 corresponds to that located at positions from 470 to 489 or at positions from 12 –31 in SEQ ID NO: 14. These results indicate that enzyme Q36 has the amino acid sequence of SEQ ID NO: 4, and it is encoded by the DNA having the base sequence as shown in SEQ ID NO: 3.

Example 5

Preparation of recombinant enzyme

In 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid nutrient culture medium (pH 7.0) consisting of 2.0 w/v % "PINE-DEX#4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, Japan, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate, and to each flask was added 50 μg/ml ampicillin and autoclaved at 120° C. for 20 min. Thereafter, the flasks were cooled and inoculated with a seed culture of the transformant BMU27 obtained in Example 1-2, followed by culturing the transformant at 27° C. for 24 hours by a rotary shaker. Apart from this, 18 L of a fresh preparation of the same liquid culture medium was placed in a 30-L jar fermentor, admixed with 50 μg/ml ampicillin, sterilized at 120° C. for 20 min, cooled and inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 37° C. for 24 hours while keeping the pH at 6–8 under aeration-agitation conditions. The resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the enzyme activity to reveal that one L of the culture yielded about 3,900 units of the enzyme. The supernatant was purified by the method in Experiment 1-1 to obtain an about 67 ml aqueous solution containing an about 165 units/ml of a recombinant enzyme having a specific activity of about 290 units/mg protein.

Example 6

Preparation of recombinant enzyme

Recombinant BRT32 obtained by the method in Experiment 3-2 was cultured similarly as in Example 5, and the resultant culture was treated with an ultrasonic integrator to disrupt cells. The resultant suspension was centrifuged to remove insoluble substances, and the resultant supernatant was assayed for the enzyme activity to have an activity of about 4,000 units per L. The supernatant was purified by the method in Experiment 1-1 to obtain an about 55 ml aqueous solution containing about 200 units/ml of a recombinant enzyme with a specific activity of about 420 units/mg protein.

Example 7

Conversion of non-reducing saccharide by recombinant enzyme

Example 7-1 (a)

Preparation of non-reducing saccharide-forming enzyme

To 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid nutrient culture medium (pH 7.0) consisting of 2.0 w/v % maltose, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate, and the flasks were autoclaved at 120° C. for 20 min. Thereafter, the flasks were cooled and inoculated with a seed culture of *Rhizobium* sp. M-11, followed by culturing it at 27° C. for 24 hours by a rotary shaker. Apart from this, 20 L of a fresh preparation of the same liquid culture medium was placed in a 30-L jar fermentor, and sterilized, inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 30° C. and at a pH of 7–8 for 24 hours under aeration-agitation conditions. Thereafter, the resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances and purified according to the method in Experiment 1-1 to obtain a non-reducing saccharide-forming enzyme having a specific activity of about 195 units/mg protein in a yield of about 220 units per L of the culture.

Throughout the specification the activity of a non-reducing saccharide-forming enzyme is expressed by the value measured on the following assay: Place 4 ml of 50 mM phosphate buffer (pH 7.0) containing 1.25 w/v % maltopentaose in a test tube, add one ml of an enzyme solution to the test tube, and incubate the solution at 40° C. for 60 min to effect enzymatic reaction. Thereafter, the reaction mixture is heated at 100° C. for 10 min to suspend the enzymatic reaction, followed by diluting it with distilled water by 10 times and assaying the reducing activity by the Somogyi-Nelson's method. One unit activity of the non-reducing saccharide-forming enzyme is defined as the amount of enzyme which decreases the reducing power corresponding to one μmol maltopentaose per min under the above conditions.

Example 7-1(b)

Preparation of syrupy product containing trehalose

A potato starch was suspended in water to give a 15 w/w % suspension which was then mixed with 0.1 w/w % calcium carbonate. The mixture was adjusted its pH to 6.0, mixed with 0.2 w/w %, d.s.b., of "TERMAMYL 60L", an α-amylase specimen commercialized by Novo Nordisk Bioindustri A/S, Copenhagen, Denmark, and enzymatically reacted at 95° C. for 15 min to effect gelatinization and liquefaction. The liquefied solution was autoclaved at 120° C. for 30 min to inactivate the remaining enzyme, rapidly cooled to 45° C., 1,000 units/g starch, d.s.b., of pullulanase commercialized by Hayashibara Biochemical Laboratories., Inc., Okayama, Japan, 3.4 units/g starch, d.s.b., of the non-reducing saccharide-forming enzyme obtained in Example 7-1(a), and 4.2 units/g starch, d.s.b., of the recombinant enzyme obtained by the method in Example 5, followed the enzymatic reaction for 48 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated to obtain a syrupy product with a concentration of about 60 w/w % in a yield of about 92%, d.s.b.

Analysis of the syrup by the method of Experiment 2-1 revealed that it contained 70.2 w/w % trehalose, 2.4 w/w % α-glucosyltrehalose, 3.3 w/w % α-maltosyltrehalose, 0.7 w/w % glucose, 10.1 w/w % maltose, 12.9 w/w % maltotriose, and 0.4 w/w % maltooligosaccharides having a degree of glucose polymerization of 4 or higher. The product, having a mild and moderate sweetness as well as an adequate viscosity and moisture-retaining ability, can be satisfactorily used in food products in general, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

Example 7-1(c)

Preparation of powdery product containing trehalose

To 4 jacketed-stainless steel columns, having a diameter of 5.4 cm and a length of 5 m each was packed homogeneity with "XT-1016 (Na^+-form)", a strong-acid cation exchange resin commercialized by Tokyo Organic Chemical Industries, Ltd., Tokyo, Japan, and the columns were cascaded in series to give a total column length of 20 m. The syrupy product obtained in Example 7-1(b) was fed to the columns at a rate of about 5 v/v % against the resin at an inner column temperature of 55° C., and the columns were fed with 55° C. hot water at an SV (space velocity) 0.3 to fractionate saccharides in the syrupy product. Based on the analysis of the saccharide composition of the eluate, fractions rich in trehalose were collected, pooled, concentrated, dried in vacuo and pulverized to obtain a solid product containing about 97 w/w % trehalose in a yield of about 56% against the starting material, d.s.b.

The product, having a mild sweetness and substantially free of reducibility, can be satisfactorily used in food products in general, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

Example 7-1(d)

Preparation of powdery crystalline trehalose

A portion of the trehalose rich fraction obtained in Example 7-1(c) was concentrated into an about 75 w/w % solution which was then transferred to a crystallizer, admixed with about 2 w/w %, d.s.b., hydrous crystalline trehalose as a seed crystal, and crystallized under gentle stirring conditions to obtain a massecuite with a crystallinity of about 45 w/w %. The massecuite was sprayed downward from a nozzle, equipped at the upper part of a spraying tower at a pressure of about 150 kg/cm^2 while about 85° C. hot air was flowing downward from the upper part of the tower to

accumulate a crystalline powder on a belt conveyer provided on the basement of the tower, followed by gradually transferring it out of the tower. Thereafter, the powder was transferred to an ageing tower and aged for 10 hours to complete the crystallization and drying while an about 40° C. hot air was blowing to the contents. Thus, a powdery product containing hydrous crystalline trehalose was obtained in a yield of about 90 w/w % against the starting material, d.s.b.

The product, having a substantial non-hygroscopicity and a mild and high-quality sweetness, can be satisfactorily used in food products in general, cosmetics, pharmaceuticals and feeds as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

Example 8

Conversion of non-reducing saccharide by recombinant enzyme

Potato starch was suspended in water to give a concentration of 6 w/w %, d.s.b., and the suspension was admixed with 500 units/g starch of isoamylase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, and enzymatically reacted for 20 hours. The reaction mixture was adjusted to a pH of 6.5, autoclaved at 120° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 95° C., admixed with 0.1 w/w % per g starch, d.s.b., of "TERMAMYL 60L", an α-amylase specimen commercialized by Novo Nordisk Bioindustri A/S, Copenhagen, Denmark, and enzymatically reacted for 15 min. The reaction mixture was heated at 130° C. for 30 min to inactivate the remaining enzyme, rapidly cooled to 45° C., admixed with 4.1 units/g starch, d.s.b., of a non-reducing saccharide-forming enzyme obtained by the method in Example 7-1(a), and 4.9 units/g starch, d.s.b., of the present recombinant enzyme obtained by the method in Example 6, and enzymatically reacted for 64 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 55° C., adjusted to pH 5.0, admixed with 10 units/g starch, d.s.b., of "GLUCOZYME", a glucoamylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and enzymatically reacted for 40 hours. The reaction mixture was heated at 95° C. For 10 min to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated to obtain an about 60 w/w % syrupy product containing about 80.5 w/w % trehalose, d.s.b. The syrupy product was concentrated into an about 84 w/w % syrup which was then transferred to a crystallizer, admixed with an about 2 w/w % hydrous crystalline trehalose, d.s.b., and crystallized under gentle stirring conditions to obtain a massecuite having a crystallinity of about 45 w/w %. The massecuite was distributed to plastic plain vessels which were then allowed to stand at ambient temperature for 3 days to effect solidification and aging, followed by detaching the resultant blocks from the vessels and pulverizing the blocks with a cutter to obtain a solid product containing hydrous crystalline trehalose in a yield of about 90 w/w % against the material starch, d.s.b.

The product, which is substantially free of hygroscopicity and readily handleable, can be arbitrarily used in food products in general, cosmetics, pharmaceuticals as a sweetening agent, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

Example 9

Conversion of non-reducing saccharide by recombinant enzyme

Potato starch was suspended in water to give a concentration of 6 w/w %, d.s.b., and the suspension was admixed with 0.01 w/w % "NEO-SPITASE", α-amylase commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, adjusted to pH 6.2, and enzymatically reacted at 85°–90° C. for 20 min to gelatinize and liquefy the starch. The liquefied starch was heated at 120° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 45° C., admixed with 500 units/g starch, d.s.b., of isoamylase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, 3.2 units/g starch, d.s.b., of a non-reducing saccharide-forming enzyme obtained by the method in Example 7-1(a), and 5.0 units/g starch, d.s.b., of the present recombinant enzyme obtained by the method in Example 5, and enzymatically reacted for 48 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 55° C., adjusted to pH 5.0, admixed with 10 units/g starch, d.s.b., of "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals Ltd., Kyoto, Japan, and enzymatically reacted for 40 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, rapidly cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated to give a concentration of about 60 w/w %, d.s.b., to obtain a syrupy product containing 78.3 w/w % trehalose, d.s.b. The syrupy product was fractionated similarly as in Example 7-1(c) except for using "CG6000(Na^+)", a strong-acid cation exchange resin commercialized by Japan Organo, Co., Ltd., Tokyo, Japan, to obtain a fraction containing abut 95 w/w % trehalose, d.s.b. The fraction was concentrated to give a concentration of about 75 w/w %, d.s.b., and, similarly as in Example 8, crystallized, and the resultant massecuite in the form of block was pulverized to obtain a powdery product containing hydrous crystalline trehalose in a yield of about 70 w/w % against the material starch, d.s.b.

The product, which is substantially free of hygroscopicity and readily handleable, can be arbitrarily used in food products in general, cosmetics, pharmaceuticals as a sweetening agent, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

As is described above, the present invention is based on the finding that a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. The present invention is to explore a way to produce the enzyme in a relatively-large scale and in a considerably-high yield. The enzyme produced by the transformant according to the present invention is the one characterized by its revealed total amino acid sequence, and because of this it can be used for the preparations of trehalose which is premised on being used in food products without fear of causing side effects.

Therefore, the present invention is an useful invention which exerts the aforesaid significant action and effect as well as giving a great contribution to this field.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 18

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1767 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1767

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 1               5                  10                  15

GCA GGC ACC GTA ACG CTG CTG GCC GGC GGG GAG CGC TAC GAG ATG GGC          96
Ala Gly Thr Val Thr Leu Leu Ala Gly Gly Glu Arg Tyr Glu Met Gly
            20                  25                  30

CGC CGC CCC GGC AAC GGG CCG GCG GAC GAA GGC TGG TGG ACG GCC GCG         144
Arg Arg Pro Gly Asn Gly Pro Ala Asp Glu Gly Trp Trp Thr Ala Ala
        35                  40                  45

GAT GCA CCG ACA GGC GCG GAC GTG GAC TAC GGA TAC CTG CTC GAC GGC         192
Asp Ala Pro Thr Gly Ala Asp Val Asp Tyr Gly Tyr Leu Leu Asp Gly
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GAC GAA ATC CCG CTG CCG GAC CCC CGG ACC CGC CGC CAG CCC GAA GGC         240
Asp Glu Ile Pro Leu Pro Asp Pro Arg Thr Arg Arg Gln Pro Glu Gly
65                  70                  75                  80

GTC CAT GCC CTG TCC CGG ACC TTC GAC CCC GGC GCC CAC CGC TGG CAG         288
Val His Ala Leu Ser Arg Thr Phe Asp Pro Gly Ala His Arg Trp Gln
                85                  90                  95

GAC GCC GGG TGG CAG GGC AGG GAA CTC CAG GGC TCC GTG ATT TAC GAA         336
Asp Ala Gly Trp Gln Gly Arg Glu Leu Gln Gly Ser Val Ile Tyr Glu
            100                 105                 110

CTC CAC ATC GGA ACG TTC ACG CCG GAA GGG ACG CTG GAC GCC GCC GCG         384
Leu His Ile Gly Thr Phe Thr Pro Glu Gly Thr Leu Asp Ala Ala Ala
        115                 120                 125

GGC AAG CTG GAC TAC CTC GCC GGC CTG GGC ATC GAC TTC ATT GAG CTG         432
Gly Lys Leu Asp Tyr Leu Ala Gly Leu Gly Ile Asp Phe Ile Glu Leu
    130                 135                 140

CTG CCC GTG AAT GCC TTC AAC GGC ACG CAC AAC TGG GGC TAC GAC GGC         480
Leu Pro Val Asn Ala Phe Asn Gly Thr His Asn Trp Gly Tyr Asp Gly
145                 150                 155                 160

GTC CAG TGG TTT GCC GTG CAT GAA GGC TAC GGC GGG CCT GCG GCG TAC         528
Val Gln Trp Phe Ala Val His Glu Gly Tyr Gly Gly Pro Ala Ala Tyr
                165                 170                 175

CAG CGG TTC GTG GAT GCG GCC CAC GCG GCC GGC CTC GGC GTC ATC CAG         576
Gln Arg Phe Val Asp Ala Ala His Ala Ala Gly Leu Gly Val Ile Gln
            180                 185                 190

GAC GTG GTC TAC AAC CAC CTC GGG CCG AGC GGG AAC TAC CTC CCC AGG         624
Asp Val Val Tyr Asn His Leu Gly Pro Ser Gly Asn Tyr Leu Pro Arg
        195                 200                 205

TAC GGC CCG TAC CTC AAG CAC GGC GAA GGC AAC ACC TGG GGC GAT TCG         672
Tyr Gly Pro Tyr Leu Lys His Gly Glu Gly Asn Thr Trp Gly Asp Ser
    210                 215                 220
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GTC Val 225	AAC Asn	CTG Leu	GAC Asp	GGG Gly	CCG Pro 230	GGA Gly	TCC Ser	GAC Asp	CAC His	GTC Val 235	CGC Arg	CAG Gln	TAC Tyr	ATC Ile	CTG Leu 240	720
GAC Asp	AAC Asn	GTG Val	GCC Ala	ATG Met 245	TGG Trp	CTG Leu	CGC Arg	GAC Asp	TAC Tyr 250	CGG Arg	GTG Val	GAC Asp	GGC Gly	CTC Leu 255	CGC Arg	768
CTG Leu	GAC Asp	GCC Ala	GTC Val 260	CAC His	GCC Ala	CTG Leu	AAG Lys	GAT Asp 265	GAG Glu	CGG Arg	GCC Ala	GTC Val	CAC His 270	ATC Ile	CTG Leu	816
GAG Glu	GAG Glu	TTC Phe 275	GGC Gly	GCG Ala	CTG Leu	GCG Ala	GAC Asp 280	GCC Ala	CTG Leu	TCG Ser	TCC Ser	GAA Glu 285	GGC Gly	GGC Gly	CGC Arg	864
CCG Pro	CTG Leu 290	ACC Thr	CTC Leu	ATC Ile	GCC Ala	GAG Glu 295	TCC Ser	GAC Asp	CTC Leu	AAC Asn	AAT Asn 300	CCG Pro	CGG Arg	CTG Leu	CTG Leu	912
TAC Tyr 305	CCC Pro	CGG Arg	GAT Asp	GTC Val	AAC Asn 310	GGC Gly	TAC Tyr	GGA Gly	CTG Leu	GCC Ala 315	GGC Gly	CAG Gln	TGG Trp	AGC Ser	GAC Asp 320	960
GAC Asp	TTC Phe	CAC His	CAC His	GCC Ala 325	GTG Val	CAC His	GTC Val	AAC Asn	GTC Val 330	AGC Ser	GGG Gly	GAA Glu	ACC Thr	ACC Thr 335	GGC Gly	1008
TAC Tyr	TAC Tyr	AGC Ser	GAC Asp 340	TTC Phe	GAC Asp	TCG Ser	CTC Leu	GGA Gly 345	GCC Ala	CTC Leu	GCC Ala	AAG Lys	GTC Val 350	CTG Leu	CGT Arg	1056
GAC Asp	GGG Gly	TTC Phe 355	TTC Phe	CAC His	GAC Asp	GGC Gly	AGC Ser 360	TAC Tyr	TCC Ser	AGC Ser	TTC Phe	CGC Arg 365	GGC Gly	CGC Arg	TGC Cys	1104
CAC His	GGC Gly 370	CGG Arg	CCG Pro	ATC Ile	AAC Asn	TTC Phe 375	AGC Ser	GCC Ala	GTG Val	CAT His	CCG Pro 380	GCC Ala	GCG Ala	CTG Leu	GTG Val	1152
GTC Val 385	TGC Cys	TCA Ser	CAG Gln	AAC Asn	CAT His 390	GAC Asp	CAG Gln	ATC Ile	GGC Gly	AAC Asn 395	CGG Arg	GCC Ala	ACC Thr	GGG Gly	GAC Asp 400	1200
CGG Arg	CTG Leu	TCC Ser	CAG Gln	TCA Ser 405	CTT Leu	CCG Pro	TAC Tyr	GGC Gly	AGC Ser 410	CTG Leu	GCC Ala	CTG Leu	GCC Ala	GCC Ala 415	GTG Val	1248
CTG Leu	ACC Thr	CTC Leu	ACC Thr 420	GGT Gly	CCG Pro	TTC Phe	ACG Thr	CCC Pro 425	ATG Met	CTG Leu	TTC Phe	ATG Met	GGA Gly 430	GAG Glu	GAA Glu	1296
TAC Tyr	GGG Gly	GCC Ala 435	ACC Thr	ACC Thr	CCG Pro	TGG Trp	CAG Gln 440	TTC Phe	TTC Phe	ACC Thr	TCG Ser	CAC His 445	CCT Pro	GAA Glu	CCC Pro	1344
GAG Glu	CTG Leu 450	GGC Gly	AAG Lys	GCC Ala	ACG Thr	GCC Ala 455	GAG Glu	GGC Gly	AGG Arg	ATC Ile	AGG Arg 460	GAG Glu	TTC Phe	GAG Glu	CGC Arg	1392
ATG Met 465	GGG Gly	TGG Trp	GAT Asp	CCC Pro	GCC Ala 470	GTC Val	GTG Val	CCC Pro	GAT Asp	CCG Pro 475	CAG Gln	GAT Asp	CCG Pro	GAG Glu	ACC Thr 480	1440
TTC Phe	ACC Thr	CGC Arg	TCC Ser	AAA Lys 485	CTG Leu	GAC Asp	TGG Trp	GCG Ala	GAA Glu 490	GCG Ala	TCC Ser	GCC Ala	GGC Gly	GAT Asp 495	CAT His	1488
GCC Ala	CGC Arg	CTC Leu	CTG Leu 500	GAG Glu	CTG Leu	TAC Tyr	CGC Arg	TCG Ser 505	CTT Leu	ATC Ile	ACG Thr	CTG Leu	CGG Arg 510	CGG Arg	TCA Ser	1536
ACT Thr	CCG Pro	GAG Glu 515	CTC Leu	GCG Ala	CGC Arg	CTG Leu	GGC Gly 520	TTT Phe	GCG Ala	GAC Asp	ACC Thr	GCC Ala 525	GTC Val	GAG Glu	TTC Phe	1584
GAC Asp	GAC Asp 530	GAC Asp	GCC Ala	CGC Arg	TGG Trp	CTC Leu 535	CGT Arg	TAT Tyr	TGG Trp	CGC Arg	GGA Gly 540	GGC Gly	GTG Val	CAG Gln	GTG Val	1632

-continued

GTG	CTG	AAC	TTC	GCG	GAC	CGT	CCC	ATC	AGC	CTG	GAC	CGG	CCG	GGA	ACC	1680
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545					550					555					560	
GCG	CTG	CTG	CTC	GCC	ACC	GAC	GAC	GCC	GTC	CGG	ATG	GAC	GGA	GTC	CAG	1728
Ala	Leu	Leu	Leu	Ala	Thr	Asp	Asp	Ala	Val	Arg	Met	Asp	Gly	Val	Gln	
				565					570					575		
GTG	GAG	CTG	CCG	CCG	CTG	AGC	GCC	GCG	GTT	CTG	CGC	GAC				1767
Val	Glu	Leu	Pro	Pro	Leu	Ser	Ala	Ala	Val	Leu	Arg	Asp				
			580					585								

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 589 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala	Lys	Pro	Val	Gln	Gly	Ala	Gly	Arg	Phe	Asp	Ile	Trp	Ala	Pro	Glu	
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			20					25					30			
Arg	Arg	Pro	Gly	Asn	Gly	Pro	Ala	Asp	Glu	Gly	Trp	Trp	Thr	Ala	Ala	
		35					40					45				
Asp	Ala	Pro	Thr	Gly	Ala	Asp	Val	Asp	Tyr	Gly	Tyr	Leu	Leu	Asp	Gly	
	50					55					60					
Asp	Glu	Ile	Pro	Leu	Pro	Asp	Pro	Arg	Thr	Arg	Arg	Gln	Pro	Glu	Gly	
65					70					75					80	
Val	His	Ala	Leu	Ser	Arg	Thr	Phe	Asp	Pro	Gly	Ala	His	Arg	Trp	Gln	
				85					90					95		
Asp	Ala	Gly	Trp	Gln	Gly	Arg	Glu	Leu	Gln	Gly	Ser	Val	Ile	Tyr	Glu	
			100					105					110			
Leu	His	Ile	Gly	Thr	Phe	Thr	Pro	Glu	Gly	Thr	Leu	Asp	Ala	Ala	Ala	
		115					120					125				
Gly	Lys	Leu	Asp	Tyr	Leu	Ala	Gly	Leu	Gly	Ile	Asp	Phe	Ile	Glu	Leu	
	130					135					140					
Leu	Pro	Val	Asn	Ala	Phe	Asn	Gly	Thr	His	Asn	Trp	Gly	Tyr	Asp	Gly	
145					150					155					160	
Val	Gln	Trp	Phe	Ala	Val	His	Glu	Gly	Tyr	Gly	Gly	Pro	Ala	Ala	Tyr	
				165					170					175		
Gln	Arg	Phe	Val	Asp	Ala	Ala	His	Ala	Ala	Gly	Leu	Gly	Val	Ile	Gln	
			180					185					190			
Asp	Val	Val	Tyr	Asn	His	Leu	Gly	Pro	Ser	Gly	Asn	Tyr	Leu	Pro	Arg	
		195					200					205				
Tyr	Gly	Pro	Tyr	Leu	Lys	His	Gly	Glu	Gly	Asn	Thr	Trp	Gly	Asp	Ser	
	210					215					220					
Val	Asn	Leu	Asp	Gly	Pro	Gly	Ser	Asp	His	Val	Arg	Gln	Tyr	Ile	Leu	
225					230					235					240	
Asp	Asn	Val	Ala	Met	Trp	Leu	Arg	Asp	Tyr	Arg	Val	Asp	Gly	Leu	Arg	
				245					250					255		
Leu	Asp	Ala	Val	His	Ala	Leu	Lys	Asp	Glu	Arg	Ala	Val	His	Ile	Leu	
			260					265					270			
Glu	Glu	Phe	Gly	Ala	Leu	Ala	Asp	Ala	Leu	Ser	Ser	Glu	Gly	Gly	Arg	
		275					280					285				
Pro	Leu	Thr	Leu	Ile	Ala	Glu	Ser	Asp	Leu	Asn	Asn	Pro	Arg	Leu	Leu	
	290					295					300					

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Tyr	Pro	Arg	Asp	Val	Asn	Gly	Tyr	Gly	Leu	Ala	Gly	Gln	Trp	Ser	Asp
305					310					315					320
Asp	Phe	His	His	Ala	Val	His	Val	Asn	Val	Ser	Gly	Glu	Thr	Thr	Gly
				325					330					335	
Tyr	Tyr	Ser	Asp	Phe	Asp	Ser	Leu	Gly	Ala	Leu	Ala	Lys	Val	Leu	Arg
			340					345					350		
Asp	Gly	Phe	Phe	His	Asp	Gly	Ser	Tyr	Ser	Ser	Phe	Arg	Gly	Arg	Cys
		355					360					365			
His	Gly	Arg	Pro	Ile	Asn	Phe	Ser	Ala	Val	His	Pro	Ala	Ala	Leu	Val
	370					375					380				
Val	Cys	Ser	Gln	Asn	His	Asp	Gln	Ile	Gly	Asn	Arg	Ala	Thr	Gly	Asp
385					390					395					400
Arg	Leu	Ser	Gln	Ser	Leu	Pro	Tyr	Gly	Ser	Leu	Ala	Leu	Ala	Ala	Val
			405						410					415	
Leu	Thr	Leu	Thr	Gly	Pro	Phe	Thr	Pro	Met	Leu	Phe	Met	Gly	Glu	Glu
			420					425					430		
Tyr	Gly	Ala	Thr	Thr	Pro	Trp	Gln	Phe	Phe	Thr	Ser	His	Pro	Glu	Pro
		435					440					445			
Glu	Leu	Gly	Lys	Ala	Thr	Ala	Glu	Gly	Arg	Ile	Arg	Glu	Phe	Glu	Arg
	450					455					460				
Met	Gly	Trp	Asp	Pro	Ala	Val	Val	Pro	Asp	Pro	Gln	Asp	Pro	Glu	Thr
465					470					475					480
Phe	Thr	Arg	Ser	Lys	Leu	Asp	Trp	Ala	Glu	Ala	Ser	Ala	Gly	Asp	His
				485					490					495	
Ala	Arg	Leu	Leu	Glu	Leu	Tyr	Arg	Ser	Leu	Ile	Thr	Leu	Arg	Arg	Ser
			500					505					510		
Thr	Pro	Glu	Leu	Ala	Arg	Leu	Gly	Phe	Ala	Asp	Thr	Ala	Val	Glu	Phe
		515					520					525			
Asp	Asp	Asp	Ala	Arg	Trp	Leu	Arg	Tyr	Trp	Arg	Gly	Gly	Val	Gln	Val
	530					535					540				
Val	Leu	Asn	Phe	Ala	Asp	Arg	Pro	Ile	Ser	Leu	Asp	Arg	Pro	Gly	Thr
545					550					555					560
Ala	Leu	Leu	Leu	Ala	Thr	Asp	Asp	Ala	Val	Arg	Met	Asp	Gly	Val	Gln
				565					570					575	
Val	Glu	Leu	Pro	Pro	Leu	Ser	Ala	Ala	Val	Leu	Arg	Asp			
			580					585							

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1791 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1791

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACG	CAC	ACC	TAC	CCG	CGG	GAA	GCC	GCG	AAA	CCC	GTC	CTG	GGC	CCC	GCA	48
Thr	His	Thr	Tyr	Pro	Arg	Glu	Ala	Ala	Lys	Pro	Val	Leu	Gly	Pro	Ala	
590					595					600					605	
CGC	TAC	GAC	GTC	TGG	GCG	CCC	AAC	GCT	GAA	TCC	GTG	ACG	CTG	CTG	GCC	96
Arg	Tyr	Asp	Val	Trp	Ala	Pro	Asn	Ala	Glu	Ser	Val	Thr	Leu	Leu	Ala	
				610					615					620		

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GGC GGG GAG CGC TAC GCC ATG CAG CGC CGG GCC GAG ACC GGG CCG GAG                144
Gly Gly Glu Arg Tyr Ala Met Gln Arg Arg Ala Glu Thr Gly Pro Glu
                625                 630                 635

GAC GCC GGC TGG TGG ACC GCC GCC GGC GCG CCT ACG GAT GGC AAC GTG                192
Asp Ala Gly Trp Trp Thr Ala Ala Gly Ala Pro Thr Asp Gly Asn Val
            640                 645                 650

GAC TAC GGG TAC CTT CTG GAC GGC GAC GAA ACA CCG CTT CCG GAT CCA                240
Asp Tyr Gly Tyr Leu Leu Asp Gly Asp Glu Thr Pro Leu Pro Asp Pro
        655                 660                 665

CGG ACC CGC CGC CAG CCC GAC GGC GTC CAC GCC CTG TCC CGC ACG TTC                288
Arg Thr Arg Arg Gln Pro Asp Gly Val His Ala Leu Ser Arg Thr Phe
    670                 675                 680                 685

GAC CCG TCC GCG TAC AGC TGG CAG GAC GAC GCC TGG CAG GGC AGG GAA                336
Asp Pro Ser Ala Tyr Ser Trp Gln Asp Asp Ala Trp Gln Gly Arg Glu
                690                 695                 700

CTG CAG GGC GCC GTC ATC TAC GAG CTC CAC CTC GGA ACA TTC ACG CCC                384
Leu Gln Gly Ala Val Ile Tyr Glu Leu His Leu Gly Thr Phe Thr Pro
            705                 710                 715

GAA GGG ACG CTG GAG GCG GCC GCC GGA AAG CTG GAC TAC CTC GCC GGC                432
Glu Gly Thr Leu Glu Ala Ala Ala Gly Lys Leu Asp Tyr Leu Ala Gly
        720                 725                 730

TTG GGC GTC GAC TTC ATC GAG CTG CTG CCG GTG AAC GCT TTC AAC GGC                480
Leu Gly Val Asp Phe Ile Glu Leu Leu Pro Val Asn Ala Phe Asn Gly
    735                 740                 745

ACG CAC AAC TGG GGT TAC GAC GGT GTC CAG TGG TTC GCT GTG CAC GAG                528
Thr His Asn Trp Gly Tyr Asp Gly Val Gln Trp Phe Ala Val His Glu
750                 755                 760                 765

GCA TAC GGC GGG CCG GAA GCG TAC CAG CGG TTC GTC GAC GCC GCC CAC                576
Ala Tyr Gly Gly Pro Glu Ala Tyr Gln Arg Phe Val Asp Ala Ala His
                770                 775                 780

GCC GCA GGC CTT GGC GTG ATC CAG GAC GTG GTC TAC AAC CAC CTC GGC                624
Ala Ala Gly Leu Gly Val Ile Gln Asp Val Val Tyr Asn His Leu Gly
            785                 790                 795

CCC AGC GGG AAC TAC CTG CCG CGG TTC GGG CCG TAC CTC AAG CAG GGC                672
Pro Ser Gly Asn Tyr Leu Pro Arg Phe Gly Pro Tyr Leu Lys Gln Gly
        800                 805                 810

GAG GGT AAC ACG TGG GGC GAC TCG GTG AAC CTG GAC GGG CCC GGC TCC                720
Glu Gly Asn Thr Trp Gly Asp Ser Val Asn Leu Asp Gly Pro Gly Ser
    815                 820                 825

GAC CAT GTG CGC CGG TAC ATC CTG GAC AAC CTG GCC ATG TGG CTG CGT                768
Asp His Val Arg Arg Tyr Ile Leu Asp Asn Leu Ala Met Trp Leu Arg
830                 835                 840                 845

GAC TAC CGG GTG GAC GGC CTG CGG CTG GAC GCC GTC CAC GCC CTG AAG                816
Asp Tyr Arg Val Asp Gly Leu Arg Leu Asp Ala Val His Ala Leu Lys
                850                 855                 860

GAT GAG CGG GCG GTG CAC ATC CTG GAG GAC TTC GGG GCG CTG GCC GAT                864
Asp Glu Arg Ala Val His Ile Leu Glu Asp Phe Gly Ala Leu Ala Asp
            865                 870                 875

CAG ATC TCC GCC GAG GTG GGA CGG CCG CTG ACG CTC ATC GCC GAG TCC                912
Gln Ile Ser Ala Glu Val Gly Arg Pro Leu Thr Leu Ile Ala Glu Ser
        880                 885                 890

GAC CTC AAC AAC CCG CGG CTG CTG TAC CCG CGG GAC GTC AAC GGG TAC                960
Asp Leu Asn Asn Pro Arg Leu Leu Tyr Pro Arg Asp Val Asn Gly Tyr
    895                 900                 905

GGG CTG GAA GGG CAG TGG AGC GAC GAC TTC CAC CAC GCC GTC CAC GTC               1008
Gly Leu Glu Gly Gln Trp Ser Asp Asp Phe His His Ala Val His Val
910                 915                 920                 925

AAC GTC ACC GGC GAA ACC ACC GGC TAC TAC AGT GAC TTC GAC TCG CTG               1056
Asn Val Thr Gly Glu Thr Thr Gly Tyr Tyr Ser Asp Phe Asp Ser Leu
                930                 935                 940
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GCC GCC CTC GCC AAG GTG CTC CGG GAC GGC TTC TTC CAC GAC GGC AGC          1104
Ala Ala Leu Ala Lys Val Leu Arg Asp Gly Phe Phe His Asp Gly Ser
        945                 950                 955

TAC TCC AGC TTC CGG GAA CGC CAC CAC GGA CGG CCG ATT AAT TTC AGC          1152
Tyr Ser Ser Phe Arg Glu Arg His His Gly Arg Pro Ile Asn Phe Ser
        960                 965                 970

GCC GTA CAC CCA GCC GCC CTG GTG GTC TGT TCG CAG AAC CAC GAC CAG          1200
Ala Val His Pro Ala Ala Leu Val Val Cys Ser Gln Asn His Asp Gln
        975                 980                 985

ATC GGC AAC CGT GCC ACG GGG GAC CGG CTC TCC CAG ACC CTG CCG TAC          1248
Ile Gly Asn Arg Ala Thr Gly Asp Arg Leu Ser Gln Thr Leu Pro Tyr
    990                 995                1000                1005

GGA AGC CTG GCC CTC GCT GCG GTG CTG ACC CTG ACG GGA CCC TTC ACG          1296
Gly Ser Leu Ala Leu Ala Ala Val Leu Thr Leu Thr Gly Pro Phe Thr
                1010                1015                1020

CCC ATG CTG CTC ATG GGC GAG GAG TAC GGC GCC AGC ACG CCG TGG CAG          1344
Pro Met Leu Leu Met Gly Glu Glu Tyr Gly Ala Ser Thr Pro Trp Gln
            1025                1030                1035

TTT TTC ACC TCG CAC CCG GAG CCG GAG CTC GGC AAG GCC ACC GCG GAG          1392
Phe Phe Thr Ser His Pro Glu Pro Glu Leu Gly Lys Ala Thr Ala Glu
        1040                1045                1050

GGC CGG ATC AAG GAG TTC GAG CGC ATG GGG TGG GAT CCC GCC GTC GTG          1440
Gly Arg Ile Lys Glu Phe Glu Arg Met Gly Trp Asp Pro Ala Val Val
    1055                1060                1065

CCC GAT CCC CAG GAT CCT GAG ACG TTC CGC CGG TCC AAG CTG GAC TGG          1488
Pro Asp Pro Gln Asp Pro Glu Thr Phe Arg Arg Ser Lys Leu Asp Trp
1070                1075                1080                1085

GCG GAA GCC GCC GAA GGC GAC CAT GCC CGG CTG CTG GAG CTG TAC CGT          1536
Ala Glu Ala Ala Glu Gly Asp His Ala Arg Leu Leu Glu Leu Tyr Arg
                1090                1095                1100

TCG CTC ACC GCC CTG CGC CGC TCC ACG CCG GAC CTC ACC AAG CTG GGC          1584
Ser Leu Thr Ala Leu Arg Arg Ser Thr Pro Asp Leu Thr Lys Leu Gly
            1105                1110                1115

TTC GAG GAC ACG CAG GTG GCG TTC GAC GAG GAC GCC CGC TGG CTG CGG          1632
Phe Glu Asp Thr Gln Val Ala Phe Asp Glu Asp Ala Arg Trp Leu Arg
        1120                1125                1130

TTC CGC CGG GGT GGC GTG CAG GTG CTG CTC AAC TTC TCG GAA CAG CCC          1680
Phe Arg Arg Gly Gly Val Gln Val Leu Leu Asn Phe Ser Glu Gln Pro
    1135                1140                1145

GTG AGC CTG GAC GGG GCG GGC ACG GCC CTG CTG CTG GCC ACC GAC GAC          1728
Val Ser Leu Asp Gly Ala Gly Thr Ala Leu Leu Leu Ala Thr Asp Asp
1150                1155                1160                1165

GCC GTC CGG CTA GAA GGT GAG CGT GCG GAA CTC GGT CCG CTG AGC GCC          1776
Ala Val Arg Leu Glu Gly Glu Arg Ala Glu Leu Gly Pro Leu Ser Ala
                1170                1175                1180

GCC GTC GTC AGC GAC                                                     1791
Ala Val Val Ser Asp
            1185
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 597 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Thr His Thr Tyr Pro Arg Glu Ala Ala Lys Pro Val Leu Gly Pro Ala
  1               5                  10                  15

Arg Tyr Asp Val Trp Ala Pro Asn Ala Glu Ser Val Thr Leu Leu Ala
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Gly Gly Glu Arg Tyr Ala Met Gln Arg Arg Ala Glu Thr Gly Pro Glu
35 40 45

Asp Ala Gly Trp Trp Thr Ala Ala Gly Ala Pro Thr Asp Gly Asn Val
50 55 60

Asp Tyr Gly Tyr Leu Leu Asp Gly Asp Glu Thr Pro Leu Pro Asp Pro
65 70 75 80

Arg Thr Arg Arg Gln Pro Asp Gly Val His Ala Leu Ser Arg Thr Phe
85 90 95

Asp Pro Ser Ala Tyr Ser Trp Gln Asp Asp Ala Trp Gln Gly Arg Glu
100 105 110

Leu Gln Gly Ala Val Ile Tyr Glu Leu His Leu Gly Thr Phe Thr Pro
115 120 125

Glu Gly Thr Leu Glu Ala Ala Ala Gly Lys Leu Asp Tyr Leu Ala Gly
130 135 140

Leu Gly Val Asp Phe Ile Glu Leu Leu Pro Val Asn Ala Phe Asn Gly
145 150 155 160

Thr His Asn Trp Gly Tyr Asp Gly Val Gln Trp Phe Ala Val His Glu
165 170 175

Ala Tyr Gly Gly Pro Glu Ala Tyr Gln Arg Phe Val Asp Ala Ala His
180 185 190

Ala Ala Gly Leu Gly Val Ile Gln Asp Val Val Tyr Asn His Leu Gly
195 200 205

Pro Ser Gly Asn Tyr Leu Pro Arg Phe Gly Pro Tyr Leu Lys Gln Gly
210 215 220

Glu Gly Asn Thr Trp Gly Asp Ser Val Asn Leu Asp Gly Pro Gly Ser
225 230 235 240

Asp His Val Arg Arg Tyr Ile Leu Asp Asn Leu Ala Met Trp Leu Arg
245 250 255

Asp Tyr Arg Val Asp Gly Leu Arg Leu Asp Ala Val His Ala Leu Lys
260 265 270

Asp Glu Arg Ala Val His Ile Leu Glu Asp Phe Gly Ala Leu Ala Asp
275 280 285

Gln Ile Ser Ala Glu Val Gly Arg Pro Leu Thr Leu Ile Ala Glu Ser
290 295 300

Asp Leu Asn Asn Pro Arg Leu Leu Tyr Pro Arg Asp Val Asn Gly Tyr
305 310 315 320

Gly Leu Glu Gly Gln Trp Ser Asp Asp Phe His His Ala Val His Val
325 330 335

Asn Val Thr Gly Glu Thr Thr Gly Tyr Tyr Ser Asp Phe Asp Ser Leu
340 345 350

Ala Ala Leu Ala Lys Val Leu Arg Asp Gly Phe Phe His Asp Gly Ser
355 360 365

Tyr Ser Ser Phe Arg Glu Arg His His Gly Arg Pro Ile Asn Phe Ser
370 375 380

Ala Val His Pro Ala Ala Leu Val Val Cys Ser Gln Asn His Asp Gln
385 390 395 400

Ile Gly Asn Arg Ala Thr Gly Asp Arg Leu Ser Gln Thr Leu Pro Tyr
405 410 415

Gly Ser Leu Ala Leu Ala Ala Val Leu Thr Leu Thr Gly Pro Phe Thr
420 425 430

Pro Met Leu Leu Met Gly Glu Glu Tyr Gly Ala Ser Thr Pro Trp Gln
435 440 445

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Phe	Phe	Thr	Ser	His	Pro	Glu	Pro	Glu	Leu	Gly	Lys	Ala	Thr	Ala	Glu	
	450					455					460					
Gly	Arg	Ile	Lys	Glu	Phe	Glu	Arg	Met	Gly	Trp	Asp	Pro	Ala	Val	Val	
465					470					475					480	
Pro	Asp	Pro	Gln	Asp	Pro	Glu	Thr	Phe	Arg	Arg	Ser	Lys	Leu	Asp	Trp	
				485					490					495		
Ala	Glu	Ala	Ala	Glu	Gly	Asp	His	Ala	Arg	Leu	Leu	Glu	Leu	Tyr	Arg	
			500					505					510			
Ser	Leu	Thr	Ala	Leu	Arg	Arg	Ser	Thr	Pro	Asp	Leu	Thr	Lys	Leu	Gly	
		515					520					525				
Phe	Glu	Asp	Thr	Gln	Val	Ala	Phe	Asp	Glu	Asp	Ala	Arg	Trp	Leu	Arg	
	530					535					540					
Phe	Arg	Arg	Gly	Gly	Val	Gln	Val	Leu	Leu	Asn	Phe	Ser	Glu	Gln	Pro	
545					550					555					560	
Val	Ser	Leu	Asp	Gly	Ala	Gly	Thr	Ala	Leu	Leu	Leu	Ala	Thr	Asp	Asp	
				565					570					575		
Ala	Val	Arg	Leu	Glu	Gly	Glu	Arg	Ala	Glu	Leu	Gly	Pro	Leu	Ser	Ala	
			580					585					590			
Ala	Val	Val	Ser	Asp												
		595														

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala	Lys	Pro	Val	Gln	Gly	Ala	Gly	Arg	Phe	Asp	Ile	Trp	Ala	Pro	Glu	
1				5					10					15		
Ala	Gly	Thr	Val													
			20													

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Thr	His	Thr	Tyr	Pro	Arg	Glu	Ala	Ala	Lys	Pro	Val	Leu	Gly	Pro	Ala	
1				5					10					15		
Arg	Tyr	Asp	Val													
			20													

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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Pro Val Gln Gly Ala Gly Arg Phe Asp Ile Trp Ala Pro Glu Ala Gly
1 5 10 15

Thr Val Thr Leu Leu
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Asp Trp Ala Glu Ala Ser Ala Gly Asp His Ala Arg Leu Leu Glu
1 5 10 15

Leu

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Glu Phe Glu Arg Met Gly Trp Asp Pro Ala Val Val Pro Asp Pro Gln
1 5 10 15

Asp Pro Glu Thr
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Pro Val Leu Gly Pro Ala Arg Tyr Asp Val Trp Ala Pro Asn Ala Glu
1 5 10 15

Ser Val Thr Leu
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2161 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 207..1994

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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GGCGCCGGGG GAGTGCTGGC GCTTGCCACC CGGCTCCCCT ACGGGCTGGA ACAGTCGGGC      60

GGCTGGCGGG ACACCGCCGT CGAGCTTGAA GCCGCCATGA CGGACGAACT GACCGGCTCC     120

ACTTTCGGGC CGGGACCGGC GGCGCTGTCA GAAGTCTTCC GGGCCTACCC GGTGGCCTTG     180

TTGGTCCCCG CGACAGGAGG CAAGTC ATG ACG CAG CCC AAC GAT GCG GCC AAG      233
                               Met Thr Gln Pro Asn Asp Ala Ala Lys
                                   600                 605

CCG GTG CAG GGA GCG GGG CGC TTC GAT ATC TGG GCG CCC GAG GCA GGC      281
Pro Val Gln Gly Ala Gly Arg Phe Asp Ile Trp Ala Pro Glu Ala Gly
               610                 615                 620

ACC GTA ACG CTG CTG GCC GGC GGG GAG CGC TAC GAG ATG GGC CGC CGC      329
Thr Val Thr Leu Leu Ala Gly Gly Glu Arg Tyr Glu Met Gly Arg Arg
           625                 630                 635

CCC GGC AAC GGG CCG GCG GAC GAA GGC TGG TGG ACG GCC GCG GAT GCA      377
Pro Gly Asn Gly Pro Ala Asp Glu Gly Trp Trp Thr Ala Ala Asp Ala
       640                 645                 650

CCG ACA GGC GCG GAC GTG GAC TAC GGA TAC CTG CTC GAC GGC GAC GAA      425
Pro Thr Gly Ala Asp Val Asp Tyr Gly Tyr Leu Leu Asp Gly Asp Glu
   655                 660                 665                 670

ATC CCG CTG CCG GAC CCC CGG ACC CGC CGC CAG CCC GAA GGC GTC CAT      473
Ile Pro Leu Pro Asp Pro Arg Thr Arg Arg Gln Pro Glu Gly Val His
               675                 680                 685

GCC CTG TCC CGG ACC TTC GAC CCC GGC GCC CAC CGC TGG CAG GAC GCC      521
Ala Leu Ser Arg Thr Phe Asp Pro Gly Ala His Arg Trp Gln Asp Ala
           690                 695                 700

GGG TGG CAG GGC AGG GAA CTC CAG GGC TCC GTG ATT TAC GAA CTC CAC      569
Gly Trp Gln Gly Arg Glu Leu Gln Gly Ser Val Ile Tyr Glu Leu His
       705                 710                 715

ATC GGA ACG TTC ACG CCG GAA GGG ACG CTG GAC GCC GCC GCG GGC AAG      617
Ile Gly Thr Phe Thr Pro Glu Gly Thr Leu Asp Ala Ala Ala Gly Lys
   720                 725                 730

CTG GAC TAC CTC GCC GGC CTG GGC ATC GAC TTC ATT GAG CTG CTG CCC      665
Leu Asp Tyr Leu Ala Gly Leu Gly Ile Asp Phe Ile Glu Leu Leu Pro
735                 740                 745                 750

GTG AAT GCC TTC AAC GGC ACG CAC AAC TGG GGC TAC GAC GGC GTC CAG      713
Val Asn Ala Phe Asn Gly Thr His Asn Trp Gly Tyr Asp Gly Val Gln
               755                 760                 765

TGG TTT GCC GTG CAT GAA GGC TAC GGC GGG CCT GCG GCG TAC CAG CGG      761
Trp Phe Ala Val His Glu Gly Tyr Gly Gly Pro Ala Ala Tyr Gln Arg
           770                 775                 780

TTC GTG GAT GCG GCC CAC GCG GCC GGC CTC GGC GTC ATC CAG GAC GTG      809
Phe Val Asp Ala Ala His Ala Ala Gly Leu Gly Val Ile Gln Asp Val
       785                 790                 795

GTC TAC AAC CAC CTC GGG CCG AGC GGG AAC TAC CTC CCC AGG TAC GGC      857
Val Tyr Asn His Leu Gly Pro Ser Gly Asn Tyr Leu Pro Arg Tyr Gly
   800                 805                 810

CCG TAC CTC AAG CAC GGC GAA GGC AAC ACC TGG GGC GAT TCG GTC AAC      905
Pro Tyr Leu Lys His Gly Glu Gly Asn Thr Trp Gly Asp Ser Val Asn
815                 820                 825                 830

CTG GAC GGG CCG GGA TCC GAC CAC GTC CGC CAG TAC ATC CTG GAC AAC      953
Leu Asp Gly Pro Gly Ser Asp His Val Arg Gln Tyr Ile Leu Asp Asn
               835                 840                 845

GTG GCC ATG TGG CTG CGC GAC TAC CGG GTG GAC GGC CTC CGC CTG GAC     1001
Val Ala Met Trp Leu Arg Asp Tyr Arg Val Asp Gly Leu Arg Leu Asp
           850                 855                 860

GCC GTC CAC GCC CTG AAG GAT GAG CGG GCC GTC CAC ATC CTG GAG GAG     1049
Ala Val His Ala Leu Lys Asp Glu Arg Ala Val His Ile Leu Glu Glu
       865                 870                 875

TTC GGC GCG CTG GCG GAC GCC CTG TCG TCC GAA GGC GGC CGC CCG CTG     1097
Phe Gly Ala Leu Ala Asp Ala Leu Ser Ser Glu Gly Gly Arg Pro Leu
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      880                 885                 890

ACC CTC ATC GCC GAG TCC GAC CTC AAC AAT CCG CGG CTG CTG TAC CCC      1145
Thr Leu Ile Ala Glu Ser Asp Leu Asn Asn Pro Arg Leu Leu Tyr Pro
895             900             905             910

CGG GAT GTC AAC GGC TAC GGA CTG GCC GGC CAG TGG AGC GAC GAC TTC      1193
Arg Asp Val Asn Gly Tyr Gly Leu Ala Gly Gln Trp Ser Asp Asp Phe
            915             920             925

CAC CAC GCC GTG CAC GTC AAC GTC AGC GGG GAA ACC ACC GGC TAC TAC      1241
His His Ala Val His Val Asn Val Ser Gly Glu Thr Thr Gly Tyr Tyr
        930             935             940

AGC GAC TTC GAC TCG CTC GGA GCC CTC GCC AAG GTC CTG CGT GAC GGG      1289
Ser Asp Phe Asp Ser Leu Gly Ala Leu Ala Lys Val Leu Arg Asp Gly
    945             950             955

TTC TTC CAC GAC GGC AGC TAC TCC AGC TTC CGC GGC CGC TGC CAC GGC      1337
Phe Phe His Asp Gly Ser Tyr Ser Ser Phe Arg Gly Arg Cys His Gly
960             965             970

CGG CCG ATC AAC TTC AGC GCC GTG CAT CCG GCC GCG CTG GTG GTC TGC      1385
Arg Pro Ile Asn Phe Ser Ala Val His Pro Ala Ala Leu Val Val Cys
975             980             985             990

TCA CAG AAC CAT GAC CAG ATC GGC AAC CGG GCC ACC GGG GAC CGG CTG      1433
Ser Gln Asn His Asp Gln Ile Gly Asn Arg Ala Thr Gly Asp Arg Leu
            995             1000            1005

TCC CAG TCA CTT CCG TAC GGC AGC CTG GCC CTG GCC GCC GTG CTG ACC      1481
Ser Gln Ser Leu Pro Tyr Gly Ser Leu Ala Leu Ala Ala Val Leu Thr
        1010            1015            1020

CTC ACC GGT CCG TTC ACG CCC ATG CTG TTC ATG GGA GAG GAA TAC GGG      1529
Leu Thr Gly Pro Phe Thr Pro Met Leu Phe Met Gly Glu Glu Tyr Gly
    1025            1030            1035

GCC ACC ACC CCG TGG CAG TTC TTC ACC TCG CAC CCT GAA CCC GAG CTG      1577
Ala Thr Thr Pro Trp Gln Phe Phe Thr Ser His Pro Glu Pro Glu Leu
1040            1045            1050

GGC AAG GCC ACG GCC GAG GGC AGG ATC AGG GAG TTC GAG CGC ATG GGG      1625
Gly Lys Ala Thr Ala Glu Gly Arg Ile Arg Glu Phe Glu Arg Met Gly
1055            1060            1065            1070

TGG GAT CCC GCC GTC GTG CCC GAT CCG CAG GAT CCG GAG ACC TTC ACC      1673
Trp Asp Pro Ala Val Val Pro Asp Pro Gln Asp Pro Glu Thr Phe Thr
            1075            1080            1085

CGC TCC AAA CTG GAC TGG GCG GAA GCG TCC GCC GGC GAT CAT GCC CGC      1721
Arg Ser Lys Leu Asp Trp Ala Glu Ala Ser Ala Gly Asp His Ala Arg
        1090            1095            1100

CTC CTG GAG CTG TAC CGC TCG CTT ATC ACG CTG CGG CGG TCA ACT CCG      1769
Leu Leu Glu Leu Tyr Arg Ser Leu Ile Thr Leu Arg Arg Ser Thr Pro
    1105            1110            1115

GAG CTC GCG CGC CTG GGC TTT GCG GAC ACC GCC GTC GAG TTC GAC GAC      1817
Glu Leu Ala Arg Leu Gly Phe Ala Asp Thr Ala Val Glu Phe Asp Asp
1120            1125            1130

GAC GCC CGC TGG CTC CGT TAT TGG CGC GGA GGC GTG CAG GTG GTG CTG      1865
Asp Ala Arg Trp Leu Arg Tyr Trp Arg Gly Gly Val Gln Val Val Leu
1135            1140            1145            1150

AAC TTC GCG GAC CGT CCC ATC AGC CTG GAC CGG CCG GGA ACC GCG CTG      1913
Asn Phe Ala Asp Arg Pro Ile Ser Leu Asp Arg Pro Gly Thr Ala Leu
            1155            1160            1165

CTG CTC GCC ACC GAC GAC GCC GTC CGG ATG GAC GGA GTC CAG GTG GAG      1961
Leu Leu Ala Thr Asp Asp Ala Val Arg Met Asp Gly Val Gln Val Glu
        1170            1175            1180

CTG CCG CCG CTG AGC GCC GCG GTT CTG CGC GAC TGAGCGTGCG CGCCTTCGGG    2014
Leu Pro Pro Leu Ser Ala Ala Val Leu Arg Asp
    1185            1190

GCGGGCGTCC TTCCGGTGAC CGGATGCTGG ACGCCCGCCC CGCAGCTCCA CAGGCGCTGG    2074
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CAGGATGGAA CGTATGACTT TTCTGGCAGC GGACAACCGC TACGAAACCA TGCCATACCG 2134

CCGCGTCGGA CGCAGCGGGC TGAAGCT 2161

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 596 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Thr Gln Pro Asn Asp Ala Ala Lys Pro Val Gln Gly Ala Gly Arg
1 5 10 15

Phe Asp Ile Trp Ala Pro Glu Ala Gly Thr Val Thr Leu Leu Ala Gly
20 25 30

Gly Glu Arg Tyr Glu Met Gly Arg Arg Pro Gly Asn Gly Pro Ala Asp
35 40 45

Glu Gly Trp Trp Thr Ala Ala Asp Ala Pro Thr Gly Ala Asp Val Asp
50 55 60

Tyr Gly Tyr Leu Leu Asp Gly Asp Glu Ile Pro Leu Pro Asp Pro Arg
65 70 75 80

Thr Arg Arg Gln Pro Glu Gly Val His Ala Leu Ser Arg Thr Phe Asp
85 90 95

Pro Gly Ala His Arg Trp Gln Asp Ala Gly Trp Gln Gly Arg Glu Leu
100 105 110

Gln Gly Ser Val Ile Tyr Glu Leu His Ile Gly Thr Phe Thr Pro Glu
115 120 125

Gly Thr Leu Asp Ala Ala Ala Gly Lys Leu Asp Tyr Leu Ala Gly Leu
130 135 140

Gly Ile Asp Phe Ile Glu Leu Leu Pro Val Asn Ala Phe Asn Gly Thr
145 150 155 160

His Asn Trp Gly Tyr Asp Gly Val Gln Trp Phe Ala Val His Glu Gly
165 170 175

Tyr Gly Gly Pro Ala Ala Tyr Gln Arg Phe Val Asp Ala Ala His Ala
180 185 190

Ala Gly Leu Gly Val Ile Gln Asp Val Val Tyr Asn His Leu Gly Pro
195 200 205

Ser Gly Asn Tyr Leu Pro Arg Tyr Gly Pro Tyr Leu Lys His Gly Glu
210 215 220

Gly Asn Thr Trp Gly Asp Ser Val Asn Leu Asp Gly Pro Gly Ser Asp
225 230 235 240

His Val Arg Gln Tyr Ile Leu Asp Asn Val Ala Met Trp Leu Arg Asp
245 250 255

Tyr Arg Val Asp Gly Leu Arg Leu Asp Ala Val His Ala Leu Lys Asp
260 265 270

Glu Arg Ala Val His Ile Leu Glu Glu Phe Gly Ala Leu Ala Asp Ala
275 280 285

Leu Ser Ser Glu Gly Gly Arg Pro Leu Thr Leu Ile Ala Glu Ser Asp
290 295 300

Leu Asn Asn Pro Arg Leu Leu Tyr Pro Arg Asp Val Asn Gly Tyr Gly
305 310 315 320

Leu Ala Gly Gln Trp Ser Asp Asp Phe His His Ala Val His Val Asn
325 330 335

Val Ser Gly Glu Thr Thr Gly Tyr Tyr Ser Asp Phe Asp Ser Leu Gly

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                340                 345                 350
Ala Leu Ala Lys Val Leu Arg Asp Gly Phe Phe His Asp Gly Ser Tyr
            355                 360                 365
Ser Ser Phe Arg Gly Arg Cys His Gly Arg Pro Ile Asn Phe Ser Ala
        370                 375                 380
Val His Pro Ala Ala Leu Val Val Cys Ser Gln Asn His Asp Gln Ile
385                 390                 395                 400
Gly Asn Arg Ala Thr Gly Asp Arg Leu Ser Gln Ser Leu Pro Tyr Gly
                405                 410                 415
Ser Leu Ala Leu Ala Ala Val Leu Thr Leu Thr Gly Pro Phe Thr Pro
            420                 425                 430
Met Leu Phe Met Gly Glu Glu Tyr Gly Ala Thr Thr Pro Trp Gln Phe
        435                 440                 445
Phe Thr Ser His Pro Glu Pro Glu Leu Gly Lys Ala Thr Ala Glu Gly
    450                 455                 460
Arg Ile Arg Glu Phe Glu Arg Met Gly Trp Asp Pro Ala Val Val Pro
465                 470                 475                 480
Asp Pro Gln Asp Pro Glu Thr Phe Thr Arg Ser Lys Leu Asp Trp Ala
                485                 490                 495
Glu Ala Ser Ala Gly Asp His Ala Arg Leu Leu Glu Leu Tyr Arg Ser
            500                 505                 510
Leu Ile Thr Leu Arg Arg Ser Thr Pro Glu Leu Ala Arg Leu Gly Phe
        515                 520                 525
Ala Asp Thr Ala Val Glu Phe Asp Asp Asp Ala Arg Trp Leu Arg Tyr
    530                 535                 540
Trp Arg Gly Gly Val Gln Val Val Leu Asn Phe Ala Asp Arg Pro Ile
545                 550                 555                 560
Ser Leu Asp Arg Pro Gly Thr Ala Leu Leu Leu Ala Thr Asp Asp Ala
                565                 570                 575
Val Arg Met Asp Gly Val Gln Val Glu Leu Pro Pro Leu Ser Ala Ala
            580                 585                 590
Val Leu Arg Asp
        595
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2056 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 90..1883

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GCCGGCTTCG GACCGGGGGC AGTGAAGATC GCCGACATCT TCCGGTCGTT CCCCGTTGCG      60

CTGCTGGTGC CGCAGACAGG AGGAGAGTC ATG ACG CAC ACC TAC CCG CGG GAA      113
                                Met Thr His Thr Tyr Pro Arg Glu
                                600

GCC GCG AAA CCC GTC CTG GGC CCC GCA CGC TAC GAC GTC TGG GCG CCC      161
Ala Ala Lys Pro Val Leu Gly Pro Ala Arg Tyr Asp Val Trp Ala Pro
605                 610                 615                 620

AAC GCT GAA TCC GTG ACG CTG CTG GCC GGC GGG GAG CGC TAC GCC ATG      209
Asn Ala Glu Ser Val Thr Leu Leu Ala Gly Gly Glu Arg Tyr Ala Met
                625                 630                 635
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CAG CGC CGG GCC GAG ACC GGG CCG GAG GAC GCC GGC TGG TGG ACC GCC      257
Gln Arg Arg Ala Glu Thr Gly Pro Glu Asp Ala Gly Trp Trp Thr Ala
                640                 645                 650

GCC GGC GCG CCT ACG GAT GGC AAC GTG GAC TAC GGG TAC CTT CTG GAC      305
Ala Gly Ala Pro Thr Asp Gly Asn Val Asp Tyr Gly Tyr Leu Leu Asp
            655                 660                 665

GGC GAC GAA ACA CCG CTT CCG GAT CCA CGG ACC CGC CGC CAG CCC GAC      353
Gly Asp Glu Thr Pro Leu Pro Asp Pro Arg Thr Arg Arg Gln Pro Asp
        670                 675                 680

GGC GTC CAC GCC CTG TCC CGC ACG TTC GAC CCG TCC GCG TAC AGC TGG      401
Gly Val His Ala Leu Ser Arg Thr Phe Asp Pro Ser Ala Tyr Ser Trp
685                 690                 695                 700

CAG GAC GAC GCC TGG CAG GGC AGG GAA CTG CAG GGC GCC GTC ATC TAC      449
Gln Asp Asp Ala Trp Gln Gly Arg Glu Leu Gln Gly Ala Val Ile Tyr
                705                 710                 715

GAG CTC CAC CTC GGA ACA TTC ACG CCC GAA GGG ACG CTG GAG GCG GCC      497
Glu Leu His Leu Gly Thr Phe Thr Pro Glu Gly Thr Leu Glu Ala Ala
            720                 725                 730

GCC GGA AAG CTG GAC TAC CTC GCC GGC TTG GGC GTC GAC TTC ATC GAG      545
Ala Gly Lys Leu Asp Tyr Leu Ala Gly Leu Gly Val Asp Phe Ile Glu
        735                 740                 745

CTG CTG CCG GTG AAC GCT TTC AAC GGC ACG CAC AAC TGG GGT TAC GAC      593
Leu Leu Pro Val Asn Ala Phe Asn Gly Thr His Asn Trp Gly Tyr Asp
750                 755                 760

GGT GTC CAG TGG TTC GCT GTG CAC GAG GCA TAC GGC GGG CCG GAA GCG      641
Gly Val Gln Trp Phe Ala Val His Glu Ala Tyr Gly Gly Pro Glu Ala
765                 770                 775                 780

TAC CAG CGG TTC GTC GAC GCC GCC CAC GCC GCA GGC CTT GGC GTG ATC      689
Tyr Gln Arg Phe Val Asp Ala Ala His Ala Ala Gly Leu Gly Val Ile
                785                 790                 795

CAG GAC GTG GTC TAC AAC CAC CTC GGC CCC AGC GGG AAC TAC CTG CCG      737
Gln Asp Val Val Tyr Asn His Leu Gly Pro Ser Gly Asn Tyr Leu Pro
            800                 805                 810

CGG TTC GGG CCG TAC CTC AAG CAG GGC GAG GGT AAC ACG TGG GGC GAC      785
Arg Phe Gly Pro Tyr Leu Lys Gln Gly Glu Gly Asn Thr Trp Gly Asp
        815                 820                 825

TCG GTG AAC CTG GAC GGG CCC GGC TCC GAC CAT GTG CGC CGG TAC ATC      833
Ser Val Asn Leu Asp Gly Pro Gly Ser Asp His Val Arg Arg Tyr Ile
830                 835                 840

CTG GAC AAC CTG GCC ATG TGG CTG CGT GAC TAC CGG GTG GAC GGC CTG      881
Leu Asp Asn Leu Ala Met Trp Leu Arg Asp Tyr Arg Val Asp Gly Leu
845                 850                 855                 860

CGG CTG GAC GCC GTC CAC GCC CTG AAG GAT GAG CGG GCG GTG CAC ATC      929
Arg Leu Asp Ala Val His Ala Leu Lys Asp Glu Arg Ala Val His Ile
                865                 870                 875

CTG GAG GAC TTC GGG GCG CTG GCC GAT CAG ATC TCC GCC GAG GTG GGA      977
Leu Glu Asp Phe Gly Ala Leu Ala Asp Gln Ile Ser Ala Glu Val Gly
            880                 885                 890

CGG CCG CTG ACG CTC ATC GCC GAG TCC GAC CTC AAC AAC CCG CGG CTG      1025
Arg Pro Leu Thr Leu Ile Ala Glu Ser Asp Leu Asn Asn Pro Arg Leu
        895                 900                 905

CTG TAC CCG CGG GAC GTC AAC GGG TAC GGG CTG GAA GGG CAG TGG AGC      1073
Leu Tyr Pro Arg Asp Val Asn Gly Tyr Gly Leu Glu Gly Gln Trp Ser
910                 915                 920

GAC GAC TTC CAC CAC GCC GTC CAC GTC AAC GTC ACC GGC GAA ACC ACC      1121
Asp Asp Phe His His Ala Val His Val Asn Val Thr Gly Glu Thr Thr
925                 930                 935                 940

GGC TAC TAC AGT GAC TTC GAC TCG CTG GCC GCC CTC GCC GCC AAG GTG CTC      1169
Gly Tyr Tyr Ser Asp Phe Asp Ser Leu Ala Ala Leu Ala Ala Lys Val Leu
                945                 950                 955
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CGG GAC GGC TTC TTC CAC GAC GGC AGC TAC TCC AGC TTC CGG GAA CGC          1217
Arg Asp Gly Phe Phe His Asp Gly Ser Tyr Ser Ser Phe Arg Glu Arg
                960                 965                 970

CAC CAC GGA CGG CCG ATT AAT TTC AGC GCC GTA CAC CCA GCC GCC CTG          1265
His His Gly Arg Pro Ile Asn Phe Ser Ala Val His Pro Ala Ala Leu
            975                 980                 985

GTG GTC TGT TCG CAG AAC CAC GAC CAG ATC GGC AAC CGT GCC ACG GGG          1313
Val Val Cys Ser Gln Asn His Asp Gln Ile Gly Asn Arg Ala Thr Gly
        990                 995                1000

GAC CGG CTC TCC CAG ACC CTG CCG TAC GGA AGC CTG GCC CTC GCT GCG          1361
Asp Arg Leu Ser Gln Thr Leu Pro Tyr Gly Ser Leu Ala Leu Ala Ala
    1005                1010                1015                1020

GTG CTG ACC CTG ACG GGA CCC TTC ACG CCC ATG CTG CTC ATG GGC GAG          1409
Val Leu Thr Leu Thr Gly Pro Phe Thr Pro Met Leu Leu Met Gly Glu
                1025                1030                1035

GAG TAC GGC GCC AGC ACG CCG TGG CAG TTT TTC ACC TCG CAC CCG GAG          1457
Glu Tyr Gly Ala Ser Thr Pro Trp Gln Phe Phe Thr Ser His Pro Glu
            1040                1045                1050

CCG GAG CTC GGC AAG GCC ACC GCG GAG GGC CGG ATC AAG GAG TTC GAG          1505
Pro Glu Leu Gly Lys Ala Thr Ala Glu Gly Arg Ile Lys Glu Phe Glu
        1055                1060                1065

CGC ATG GGG TGG GAT CCC GCC GTC GTG CCC GAT CCC CAG GAT CCT GAG          1553
Arg Met Gly Trp Asp Pro Ala Val Val Pro Asp Pro Gln Asp Pro Glu
    1070                1075                1080

ACG TTC CGC CGG TCC AAG CTG GAC TGG GCG GAA GCC GCC GAA GGC GAC          1601
Thr Phe Arg Arg Ser Lys Leu Asp Trp Ala Glu Ala Ala Glu Gly Asp
1085                1090                1095                1100

CAT GCC CGG CTG CTG GAG CTG TAC CGT TCG CTC ACC GCC CTG CGC CGC          1649
His Ala Arg Leu Leu Glu Leu Tyr Arg Ser Leu Thr Ala Leu Arg Arg
                1105                1110                1115

TCC ACG CCG GAC CTC ACC AAG CTG GGC TTC GAG GAC ACG CAG GTG GCG          1697
Ser Thr Pro Asp Leu Thr Lys Leu Gly Phe Glu Asp Thr Gln Val Ala
            1120                1125                1130

TTC GAC GAG GAC GCC CGC TGG CTG CGG TTC CGC CGG GGT GGC GTG CAG          1745
Phe Asp Glu Asp Ala Arg Trp Leu Arg Phe Arg Arg Gly Gly Val Gln
        1135                1140                1145

GTG CTG CTC AAC TTC TCG GAA CAG CCC GTG AGC CTG GAC GGG GCG GGC          1793
Val Leu Leu Asn Phe Ser Glu Gln Pro Val Ser Leu Asp Gly Ala Gly
    1150                1155                1160

ACG GCC CTG CTG CTG GCC ACC GAC GAC GCC GTC CGG CTA GAA GGT GAG          1841
Thr Ala Leu Leu Leu Ala Thr Asp Asp Ala Val Arg Leu Glu Gly Glu
1165                1170                1175                1180

CGT GCG GAA CTC GGT CCG CTG AGC GCC GCC GTC GTC AGC GAC                  1883
Arg Ala Glu Leu Gly Pro Leu Ser Ala Ala Val Val Ser Asp
                1185                1190

TGACGTTTTC TTGGGGGCGG CGTCCACCGC CGGTGACCGG ATGGTGGACG TCCGCCCCGA       1943

AGCCTCGGCG CGGCTGGCAG GATGGAACGC ATGACTTATG TGGCCTCGGA CACCCGCTAC       2003

GACACCATGC CCTACCGCCG CGTCGGACGC AGCGGCCTCA AACTGCCGGC CAT               2056
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 598 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Thr His Thr Tyr Pro Arg Glu Ala Ala Lys Pro Val Leu Gly Pro
```

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1				5				10				15			
Ala	Arg	Tyr	Asp	Val	Trp	Ala	Pro	Asn	Ala	Glu	Ser	Val	Thr	Leu	Leu
			20					25					30		
Ala	Gly	Gly	Glu	Arg	Tyr	Ala	Met	Gln	Arg	Arg	Ala	Glu	Thr	Gly	Pro
			35					40					45		
Glu	Asp	Ala	Gly	Trp	Trp	Thr	Ala	Ala	Gly	Ala	Pro	Thr	Asp	Gly	Asn
			50					55					60		
Val	Asp	Tyr	Gly	Tyr	Leu	Leu	Asp	Gly	Asp	Glu	Thr	Pro	Leu	Pro	Asp
65						70					75				80
Pro	Arg	Thr	Arg	Arg	Gln	Pro	Asp	Gly	Val	His	Ala	Leu	Ser	Arg	Thr
				85					90					95	
Phe	Asp	Pro	Ser	Ala	Tyr	Ser	Trp	Gln	Asp	Asp	Ala	Trp	Gln	Gly	Arg
			100					105					110		
Glu	Leu	Gln	Gly	Ala	Val	Ile	Tyr	Glu	Leu	His	Leu	Gly	Thr	Phe	Thr
		115					120					125			
Pro	Glu	Gly	Thr	Leu	Glu	Ala	Ala	Ala	Gly	Lys	Leu	Asp	Tyr	Leu	Ala
	130					135					140				
Gly	Leu	Gly	Val	Asp	Phe	Ile	Glu	Leu	Leu	Pro	Val	Asn	Ala	Phe	Asn
145					150					155					160
Gly	Thr	His	Asn	Trp	Gly	Tyr	Asp	Gly	Val	Gln	Trp	Phe	Ala	Val	His
				165					170					175	
Glu	Ala	Tyr	Gly	Gly	Pro	Glu	Ala	Tyr	Gln	Arg	Phe	Val	Asp	Ala	Ala
			180					185					190		
His	Ala	Ala	Gly	Leu	Gly	Val	Ile	Gln	Asp	Val	Val	Tyr	Asn	His	Leu
		195					200					205			
Gly	Pro	Ser	Gly	Asn	Tyr	Leu	Pro	Arg	Phe	Gly	Pro	Tyr	Leu	Lys	Gln
	210					215					220				
Gly	Glu	Gly	Asn	Thr	Trp	Gly	Asp	Ser	Val	Asn	Leu	Asp	Gly	Pro	Gly
225					230					235					240
Ser	Asp	His	Val	Arg	Arg	Tyr	Ile	Leu	Asp	Asn	Leu	Ala	Met	Trp	Leu
				245					250					255	
Arg	Asp	Tyr	Arg	Val	Asp	Gly	Leu	Arg	Leu	Asp	Ala	Val	His	Ala	Leu
			260					265					270		
Lys	Asp	Glu	Arg	Ala	Val	His	Ile	Leu	Glu	Asp	Phe	Gly	Ala	Leu	Ala
		275					280					285			
Asp	Gln	Ile	Ser	Ala	Glu	Val	Gly	Arg	Pro	Leu	Thr	Leu	Ile	Ala	Glu
	290					295					300				
Ser	Asp	Leu	Asn	Asn	Pro	Arg	Leu	Leu	Tyr	Pro	Arg	Asp	Val	Asn	Gly
305					310					315					320
Tyr	Gly	Leu	Glu	Gly	Gln	Trp	Ser	Asp	Asp	Phe	His	His	Ala	Val	His
				325					330					335	
Val	Asn	Val	Thr	Gly	Glu	Thr	Thr	Gly	Tyr	Tyr	Ser	Asp	Phe	Asp	Ser
			340					345					350		
Leu	Ala	Ala	Leu	Ala	Lys	Val	Leu	Arg	Asp	Gly	Phe	Phe	His	Asp	Gly
		355					360					365			
Ser	Tyr	Ser	Ser	Phe	Arg	Glu	Arg	His	His	Gly	Arg	Pro	Ile	Asn	Phe
	370					375					380				
Ser	Ala	Val	His	Pro	Ala	Ala	Leu	Val	Val	Cys	Ser	Gln	Asn	His	Asp
385					390					395					400
Gln	Ile	Gly	Asn	Arg	Ala	Thr	Gly	Asp	Arg	Leu	Ser	Gln	Thr	Leu	Pro
				405					410					415	
Tyr	Gly	Ser	Leu	Ala	Leu	Ala	Ala	Val	Leu	Thr	Leu	Thr	Gly	Pro	Phe
			420					425					430		

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Thr	Pro	Met	Leu	Leu	Met	Gly	Glu	Glu	Tyr	Gly	Ala	Ser	Thr	Pro	Trp
		435					440					445			
Gln	Phe	Phe	Thr	Ser	His	Pro	Glu	Pro	Glu	Leu	Gly	Lys	Ala	Thr	Ala
	450					455					460				
Glu	Gly	Arg	Ile	Lys	Glu	Phe	Glu	Arg	Met	Gly	Trp	Asp	Pro	Ala	Val
465					470					475					480
Val	Pro	Asp	Pro	Gln	Asp	Pro	Glu	Thr	Phe	Arg	Arg	Ser	Lys	Leu	Asp
				485					490					495	
Trp	Ala	Glu	Ala	Ala	Glu	Gly	Asp	His	Ala	Arg	Leu	Leu	Glu	Leu	Tyr
			500					505					510		
Arg	Ser	Leu	Thr	Ala	Leu	Arg	Arg	Ser	Thr	Pro	Asp	Leu	Thr	Lys	Leu
		515					520					525			
Gly	Phe	Glu	Asp	Thr	Gln	Val	Ala	Phe	Asp	Glu	Asp	Ala	Arg	Trp	Leu
	530					535					540				
Arg	Phe	Arg	Arg	Gly	Gly	Val	Gln	Val	Leu	Leu	Asn	Phe	Ser	Glu	Gln
545					550					555					560
Pro	Val	Ser	Leu	Asp	Gly	Ala	Gly	Thr	Ala	Leu	Leu	Leu	Ala	Thr	Asp
				565					570					575	
Asp	Ala	Val	Arg	Leu	Glu	Gly	Glu	Arg	Ala	Glu	Leu	Gly	Pro	Leu	Ser
			580					585					590		
Ala	Ala	Val	Val	Ser	Asp										
		595													

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTYGAYATHT GGGCNCC 17

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGGGNTGGG AYCCNGC 17

(2) INFORMATION FOR SEQ ID NO:18:

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAYGAYGTNT GGGC 14

We claim:

1. A method for converting a non-reducing saccharide, comprising the step of:

introducing a recombinant DNA carrying a self-replicable vector and a nucleotide sequence coding for the expression of a variant of an enzyme of SEQ ID NO: 2 or SEQ ID NO: 4, which variant acts on a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher to release trehalose, into a host microorganism to obtain a recombinant microorganism, wherein the variant has an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 with one or more amino acid residues in SEQ ID NO: 2 or SEQ ID NO: 4 deleted, replaced with different amino acids, or supplemented with one or more amino acids without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4;

culturing said recombinant microorganism having said recombinant DNA for expression of said variant enzyme in a nutrient culture medium to accumulate said variant enzyme;

recovering said accumulated variant enzyme; and

subjecting said non-reducing saccharine to the action of said recovered variant enzyme possessing trehalose releasing activity to convert said non-reducing saccharide.

2. The method according to claim **1**, wherein said non-reducing saccharide is prepared by successively treating an amylaceous substance selected from the group consisting of starch, amylopectin, amylose, and mixtures thereof, with acid in combination with or without amylase.

3. The method according to claim **1**, wherein said non-reducing saccharide is selected from the group consisting of α-glucosyltrehalose, α-maltosyltrehalose, α-maltotriosyltrehalose, α-maltotetraosyltrehalose, α-maltopentaosyltrehalose, and mixtures thereof.

4. The method according to claim **1**, wherein said non-reducing saccharide has a concentration of 50 w/v % or lower in solution, and said subjecting step is carried out at a temperature of about 40°–55° C. and a pH in a range of about 6–8.

5.The method according to claim **1**, wherein the variant has one or more amino acid residues in SEQ ID NO: 2 or SEQ ID NO: 4 replaced with different amino acids without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4.

6.The method according to claim **1**, wherein the variant has one or more amino acid residues in SEQ ID NO: 2 or SEQ ID NO: 4 deleted without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4.

7.The method according to claim **1**, wherein the variant has one or more amino acid residues in SEQ ID NO: 2 or SEQ ID NO: 4 supplemented with one or more amino acids without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4.

* * * * *